Refine Search

Search Results -

Terms	Documents
L5 and (pro-UK)	7

Database:
US Pre-Grant Publication Full-Text Database
US Patents Full-Text Database
US OCR Full-Text Database
EPO Abstracts Database
JPO Abstracts Database
Derwent World Patents Index
IBM Technical Disclosure Bulletins

Search: L7

Refine Search

Recall Text | Clear | Interrupt

Search History

DATE: Friday, November 18, 2005 Printable Copy Create Case

Set Name side by side	Query	Hit Count	Set Name result set
DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=OR			
L7	l5 and (pro-UK)	7	L7
L6	L5 and (M5 mutant)	16	L6
L5	sarmientos.in.	175	L5
DB=USPT; PLUR=YES; OP=OR			
L4	L1 and (BL21)	0	L4
L3	L1 and (E coli B strain)	1	L3
L2	L1 and (BL21-DE3-RIL)	0	L2
L1	5472692.pn.	1	L1

END OF SEARCH HISTORY

Hit List

Clear | Generate Collection | Print | Fwd Refs | Bkwd Refs | Generate OACS

First Hit

Search Results - Record(s) 1 through 10 of 16 returned.

☐ 1. Document ID: US 20050255460 A1

Using default format because multiple data bases are involved.

L6: Entry 1 of 16 File: PGPB Nov 17, 2005

PGPUB-DOCUMENT-NUMBER: 20050255460
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20050255460 A1

TITLE: Methods of diagnosing cervical cancer

PUBLICATION-DATE: November 17, 2005

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Lu, Peter S.	Sunnyvale	CA	US
Schweizer, Johannes	Mountain View	CA	US
Diaz-Sarmiento, Chamorro Somoza	Mountain View	CA	US
Belmares, Michael P.	San Jose	CA	US

US-CL-CURRENT: 435/5

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Ima

☐ 2. Document ID: US 20050031607 A1

L6: Entry 2 of 16 File: PGPB Feb 10, 2005

PGPUB-DOCUMENT-NUMBER: 20050031607
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20050031607 A1

TITLE: Methods, devices, and compositions for lysis of occlusive blood clots while sparing wound sealing clots

PUBLICATION-DATE: February 10, 2005

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Gurewich, Victor	Cambridge	MA	US
Williams, John N.	Boston	MA	US
Liu, Jian-Ning	Brighton	MA	US
Sarmientos, Paolo	Lecco		IT
Pagani, Massimiliano	Castelli Calepio (Bergamo)		IT

US-CL-CURRENT: 424/94.64

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Ima

☐ 3. Document ID: US 20050019863 A1

L6: Entry 3 of 16 File: PGPB Jan 27, 2005

PGPUB-DOCUMENT-NUMBER: 20050019863
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20050019863 A1

TITLE: Methods of making pro-urokinase mutants

PUBLICATION-DATE: January 27, 2005

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Sarmientos, Paolo	Leoco		IT
Pagani, Massimiliano	Cividino		IT

US-CL-CURRENT: 435/69.1; 435/215, 435/252.33, 435/320.1, 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Ima

☐ 4. Document ID: US 20040229298 A1

L6: Entry 4 of 16 File: PGPB Nov 18, 2004

PGPUB-DOCUMENT-NUMBER: 20040229298
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20040229298 A1

TITLE: Methods and compositions for treating cervical cancer

PUBLICATION-DATE: November 18, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Lu, Peter S.	Mountain View	CA	US
Bagowski, Christoph Peter	Palo Alto	CA	US
Schweizer, Johannes	Mountain View	CA	US
Diaz-Sarmiento, Chamorro Somoza	Palo Alto	CA	US
Garman, Jonathan David	San Jose	CA	US
Belmares, Michael P.	San Jose	CA	US

US-CL-CURRENT: 435/7.23; 530/350

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Ima

☐ 5. Document ID: US 20040018487 A1

L6: Entry 5 of 16 File: PGPB Jan 29, 2004

PGPUB-DOCUMENT-NUMBER: 20040018487
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20040018487 A1

TITLE: Methods of diagnosing cervical cancer

PUBLICATION-DATE: January 29, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Lu, Peter S.	Mountain View	CA	US
Schweizer, Johannes	Mountain View	CA	US
Diaz-Sarmiento, Chamorro Somoza	Palo Alto	CA	US
Belmares, Michael P.	San Jose	CA	US

US-CL-CURRENT: 435/5; 536/23.72

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Ima

---

☐ 6. Document ID: US 20030049695 A1

L6: Entry 6 of 16 File: PGPB Mar 13, 2003

PGPUB-DOCUMENT-NUMBER: 20030049695
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030049695 A1

TITLE: PDZ domain interactions and lipid rafts

PUBLICATION-DATE: March 13, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Lu, Peter S.	Mountain View	CA	US
Diaz-Sarmiento, Chamorro Somoza	Palo Alto	CA	US
Seed, Brian	Boston	MA	US
Xavier, Ramnik	Boston	MA	US
Irving, Bryan Allen	San Francisco	CA	US

US-CL-CURRENT: 435/7.21

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Ima

---

☐ 7. Document ID: US 5866358 A

L6: Entry 7 of 16 File: USPT Feb 2, 1999

US-PAT-NO: 5866358
DOCUMENT-IDENTIFIER: US 5866358 A

TITLE: Production of human prourokinase

DATE-ISSUED: February 2, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Brandazza; Anna	Rivolta d'Adda			IT
Sarmientos; Paolo	Milan			IT
Orsini; Gaetano	Gallarate			IT

US-CL-CURRENT: 435/69.1; 435/215, 435/320.1, 435/71.1, 536/24.1

☐ 8. Document ID: US 5352589 A

L6: Entry 8 of 16 File: USPT Oct 4, 1994

US-PAT-NO: 5352589
DOCUMENT-IDENTIFIER: US 5352589 A

TITLE: Deletion mutant of basic fibroblast growth factor and production thereof

DATE-ISSUED: October 4, 1994

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bergonzoni; Laura	Milan			IT
Isacchi; Antonella	Milan			IT
Sarmientos; Paolo	Milan			IT
Cauet; Gilles	Buccinasco			IT

US-CL-CURRENT: 435/69.4; 435/252.33, 530/399

☐ 9. Document ID: JP 04252184 A

L6: Entry 9 of 16 File: JPAB Sep 8, 1992

PUB-NO: JP404252184A
DOCUMENT-IDENTIFIER: JP 04252184 A
TITLE: PRO-UROKINASE DERIVATIVE

PUBN-DATE: September 8, 1992

INVENTOR-INFORMATION:

NAME	COUNTRY
BRANDAZZA, ANNA	
LANSEN, JAQUELINE	
ORSINI, GAETANO	
SARMIENTOS, PAOLO	

INT-CL (IPC): C12N 9/72; A61K 37/465; A61K 37/547; C12N 1/21; C12N 15/58

☐ 10. Document ID: WO 2004093797 A2

L6: Entry 10 of 16 File: EPAB Nov 4, 2004

PUB-NO: WO2004093797A2
DOCUMENT-IDENTIFIER: WO 2004093797 A2
TITLE: METHODS, DEVICES, AND COMPOSITIONS FOR LYSIS OF OCCLUSIVE BLOOD CLOTS WHILE SPARING WOUND SEALING CLOTS

PUBN-DATE: November 4, 2004

INVENTOR-INFORMATION:

NAME	COUNTRY
GUREWICH, VICTOR	US
WILLIAMS, JOHN N	US
LIU, JIAN-NING	US
SARMIENTOS, PAOLO	US
PAGANI, MASSIMILIANO	US

INT-CL (IPC): A61 K 0/

Terms	Documents
L5 and (M5 mutant)	16

Hit List

Clear | Generate Collection | Print | Fwd Refs | Bkwd Refs | Generate OACS

First Hit

Search Results - Record(s) 1 through 7 of 7 returned.

☐ 1. Document ID: US 20050031607 A1

Using default format because multiple data bases are involved.

L7: Entry 1 of 7 File: PGPB Feb 10, 2005

PGPUB-DOCUMENT-NUMBER: 20050031607
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20050031607 A1

TITLE: Methods, devices, and compositions for lysis of occlusive blood clots while sparing wound sealing clots

PUBLICATION-DATE: February 10, 2005

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Gurewich, Victor	Cambridge	MA	US
Williams, John N.	Boston	MA	US
Liu, Jian-Ning	Brighton	MA	US
Sarmientos, Paolo	Lecco		IT
Pagani, Massimiliano	Castelli Calepio (Bergamo)		IT

US-CL-CURRENT: 424/94.64

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Ima

☐ 2. Document ID: US 20050019863 A1

L7: Entry 2 of 7 File: PGPB Jan 27, 2005

PGPUB-DOCUMENT-NUMBER: 20050019863
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20050019863 A1

TITLE: Methods of making pro-urokinase mutants

PUBLICATION-DATE: January 27, 2005

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Sarmientos, Paolo	Leoco		IT
Pagani, Massimiliano	Cividino		IT

US-CL-CURRENT: 435/69.1; 435/215, 435/252.33, 435/320.1, 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Ima

☐ 3. Document ID: US 5866358 A

L7: Entry 3 of 7 File: USPT Feb 2, 1999

US-PAT-NO: 5866358
DOCUMENT-IDENTIFIER: US 5866358 A

TITLE: Production of human prourokinase

DATE-ISSUED: February 2, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Brandazza; Anna	Rivolta d'Adda			IT
Sarmientos; Paolo	Milan			IT
Orsini; Gaetano	Gallarate			IT

US-CL-CURRENT: 435/69.1; 435/215, 435/320.1, 435/71.1, 536/24.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | | | Claims | KWIC | Draw Desc | Ima

---

☐ 4. Document ID: WO 2004093797 A2

L7: Entry 4 of 7 File: EPAB Nov 4, 2004

PUB-NO: WO2004093797A2
DOCUMENT-IDENTIFIER: WO 2004093797 A2
TITLE: METHODS, DEVICES, AND COMPOSITIONS FOR LYSIS OF OCCLUSIVE BLOOD CLOTS WHILE SPARING WOUND SEALING CLOTS

PUBN-DATE: November 4, 2004

INVENTOR-INFORMATION:

NAME	COUNTRY
GUREWICH, VICTOR	US
WILLIAMS, JOHN N	US
LIU, JIAN-NING	US
SARMIENTOS, PAOLO	US
PAGANI, MASSIMILIANO	US

INT-CL (IPC): A61 K 0/

Full | Title | Citation | Front | Review | Classification | Date | Reference | | | Claims | KWIC | Draw Desc | Ima

---

☐ 5. Document ID: US 20050031607 A1, WO 2004093797 A2, CA 2426115 A1, US 20050019863 A1

L7: Entry 5 of 7 File: DWPI Feb 10, 2005

DERWENT-ACC-NO: 2004-775860
DERWENT-WEEK: 200512
COPYRIGHT 2005 DERWENT INFORMATION LTD
TITLE: Use of pro-urokinase (pro-UK) mutant in clearing a lumen of blood clots for treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery

INVENTOR: GUREWICH, V; LIU, J ; PAGANI, M ; SARMIENTOS, P ; WILLIAMS, J N

PRIORITY-DATA: 2003US-464003P (April 18, 2003), 2003US-463930P (April 18, 2003), 2003US-464002P (April 18, 2003), 2004US-0826598 (April 16, 2004), 2004US-0826826 (April 16, 2004)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
US 20050031607 A1	February 10, 2005		000	A61K038/48
WO 2004093797 A2	November 4, 2004	E	090	A61K000/00
CA 2426115 A1	October 18, 2004	E	000	C12N009/72
US 20050019863 A1	January 27, 2005		000	C12P021/02

INT-CL (IPC): A61 K 0/00; A61 K 38/48; A61 K 38/49; A61 L 29/16; A61 P 7/02; A61 P 9/00; C07 H 21/04; C12 N 9/72; C12 P 21/02

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Ima

---

☐ 6. Document ID: DE 4122688 A, GB 2246133 A, IT 1250653 B, JP 04252185 A

L7: Entry 6 of 7 File: DWPI Jan 16, 1992

DERWENT-ACC-NO: 1992-025815
DERWENT-WEEK: 199204
COPYRIGHT 2005 DERWENT INFORMATION LTD
TITLE: New amidated derivs. of human pro-urokinase - are fibrinolytic and can be used to treat acute myocardial infarction, pulmonary embolism or deep venous thrombosis

INVENTOR: GOZZINI, L; PEREGO, R ; RONCUCCI, R ; SARMIENTOS, P ; VISCO, C

PRIORITY-DATA: 1991GB-0014846 (July 10, 1991), 1990GB-0015369 (July 12, 1990)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
DE 4122688 A	January 16, 1992		000	
GB 2246133 A	January 22, 1992		000	
IT 1250653 B	April 21, 1995		000	C12K000/00
JP 04252185 A	September 8, 1992		012	C12N009/72

INT-CL (IPC): A61K 37/465; A61K 37/54; A61K 37/547; C12K 0/00; C12N 1/00; C12N 1/21; C12N 5/16; C12N 9/72; C12N 15/66; C12R 1/12

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Ima

---

☐ 7. Document ID: EP 365894 A, US 5866358 A, CA 2000408 A, WO 9004023 A, PT 91929 A, FI 9002893 A, ZA 8907663 A, NO 9002564 A, DK 9001410 A, EP 407490 A, CN 1042181 A, AU 8943823 A, HU 55443 T, JP 03502526 W, NZ 230950 A, HU 209149 B, PH 27348 A

L7: Entry 7 of 7 File: DWPI May 2, 1990

DERWENT-ACC-NO: 1990-133447
DERWENT-WEEK: 199912
COPYRIGHT 2005 DERWENT INFORMATION LTD
TITLE: Non-glycosylated pro-urokinase prodn. - using E coli B strains and E coli promoter PTRP and Shine-Dalgarno sequence MS-2

INVENTOR: BRANDAZZA, A; ORSINI, G ; SARMIENTOS, P

PRIORITY-DATA: 1988GB-0023833 (October 11, 1988)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
EP 365894 A	May 2, 1990		021	
US 5866358 A	February 2, 1999		000	C12N001/21
CA 2000408 A	April 11, 1990		000	
WO 9004023 A	April 19, 1990		000	
PT 91929 A	April 30, 1990		000	
FI 9002893 A	June 8, 1990		000	
ZA 8907663 A	August 29, 1990		000	
NO 9002564 A	August 9, 1990		000	
DK 9001410 A	August 13, 1990		000	
EP 407490 A	January 16, 1991		000	
CN 1042181 A	May 16, 1990		000	
AU 8943823 A	May 2, 1991		000	
HU 55443 T	May 28, 1991		000	
JP 03502526 W	June 13, 1991		000	
NZ 230950 A	July 27, 1993		000	C12N015/54
HU 209149 B	March 28, 1994		000	C12N015/58
PH 27348 A	June 8, 1993		000	C12N015/58

INT-CL (IPC): C07K 7/04; C07K 15/04; C07K 15/06; C12N 1/21; C12N 9/12; C12N 9/72; C12N 15/54; C12N 15/58; C12N 15/70; C12P 21/02

Terms	Documents
L5 and (pro-UK)	7

```
Connecting via Winsock to STN


Welcome to STN International!  Enter x:x

LOGINID:SSSPTA1653HXP

PASSWORD:
TERMINAL (ENTER 1, 2, 3, OR ?):2


* * * * * * * * * *   Welcome to STN International   * * * * * * * * * *

 NEWS  1           Web Page URLs for STN Seminar Schedule - N. America
 NEWS  2           "Ask CAS" for self-help around the clock
 NEWS  3  JUL 20   Powerful new interactive analysis and visualization software,
                   STN AnaVist, now available
 NEWS  4  AUG 11   STN AnaVist workshops to be held in North America
 NEWS  5  AUG 30   CA/CAplus -Increased access to 19th century research documents
 NEWS  6  AUG 30   CASREACT - Enhanced with displayable reaction conditions
 NEWS  7  SEP 09   ACD predicted properties enhanced in REGISTRY/ZREGISTRY
 NEWS  8  OCT 03   MATHDI removed from STN
 NEWS  9  OCT 04   CA/CAplus-Canadian Intellectual Property Office (CIPO) added
                   to core patent offices
 NEWS 10  OCT 06   STN AnaVist workshops to be held in North America
 NEWS 11  OCT 13   New CAS Information Use Policies Effective October 17, 2005
 NEWS 12  OCT 17   STN(R) AnaVist(TM), Version 1.01, allows the export/download
                   of CAplus documents for use in third-party analysis and
                   visualization tools
 NEWS 13  OCT 27   Free KWIC format extended in full-text databases
 NEWS 14  OCT 27   DIOGENES content streamlined
 NEWS 15  OCT 27   EPFULL enhanced with additional content
 NEWS 16  NOV 14   CA/CAplus - Expanded coverage of German academic research

 NEWS EXPRESS  JUNE 13 CURRENT WINDOWS VERSION IS V8.0, CURRENT
               MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
               AND CURRENT DISCOVER FILE IS DATED 13 JUNE 2005

 NEWS HOURS    STN Operating Hours Plus Help Desk Availability
 NEWS INTER    General Internet Information
 NEWS LOGIN    Welcome Banner and News Items
 NEWS PHONE    Direct Dial and Telecommunication Network Access to STN
 NEWS WWW      CAS World Wide Web Site (general information)

Enter NEWS followed by the item number or name to see news on that
specific topic.

  All use of STN is subject to the provisions of the STN Customer
  agreement.  Please note that this agreement limits use to scientific
  research.  Use for software development or design or implementation
  of commercial gateways or other similar uses is prohibited and may
  result in loss of user privileges and other penalties.

  * * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 14:24:41 ON 18 NOV 2005

=> file medline, uspatful, dgene, embase, wpids, fsta, jicst, biosis, biotechds
COST IN U.S. DOLLARS                          SINCE FILE      TOTAL
                                                 ENTRY    SESSION
```

FULL ESTIMATED COST 0.21 0.21

FILE 'MEDLINE' ENTERED AT 14:25:14 ON 18 NOV 2005

FILE 'USPATFULL' ENTERED AT 14:25:14 ON 18 NOV 2005
CA INDEXING COPYRIGHT (C) 2005 AMERICAN CHEMICAL SOCIETY (ACS)

FILE 'DGENE' ENTERED AT 14:25:14 ON 18 NOV 2005
COPYRIGHT (C) 2005 THE THOMSON CORPORATION

FILE 'EMBASE' ENTERED AT 14:25:14 ON 18 NOV 2005
Copyright (c) 2005 Elsevier B.V. All rights reserved.

FILE 'WPIDS' ENTERED AT 14:25:14 ON 18 NOV 2005
COPYRIGHT (C) 2005 THE THOMSON CORPORATION

FILE 'FSTA' ENTERED AT 14:25:14 ON 18 NOV 2005
COPYRIGHT (C) 2005 International Food Information Service

FILE 'JICST-EPLUS' ENTERED AT 14:25:14 ON 18 NOV 2005
COPYRIGHT (C) 2005 Japan Science and Technology Agency (JST)

FILE 'BIOSIS' ENTERED AT 14:25:14 ON 18 NOV 2005
Copyright (c) 2005 The Thomson Corporation

FILE 'BIOTECHDS' ENTERED AT 14:25:14 ON 18 NOV 2005
COPYRIGHT (C) 2005 THE THOMSON CORPORATION

=> s (pro-urokinase mutant or pro-UK mutant)
8 FILES SEARCHED...
L1 43 (PRO-UROKINASE MUTANT OR PRO-UK MUTANT)

=> s l1 and (preparation method)
5 FILES SEARCHED...
L2 0 L1 AND (PREPARATION METHOD)

=> s l1 and M5
L3 8 L1 AND M5

=> d l3 ti abs ibib tot

L3 ANSWER 1 OF 8 USPATFULL on STN
TI Methods, devices, and compositions for lysis of occlusive blood clots while sparing wound sealing clots
AB It has now been discovered that certain mutant forms of pro-urokinase ("pro-UK"), such as so-called **pro-UK mutant "M5"** (Lys.sup.300→His), perform in the manner of pro-UK in lysing "bad" blood clots (those clots that occlude blood vessels), while sparing hemostatic fibrin in the so-called "good" blood clots (those clots that seal wounds, e.g., after surgery or other tissue injury). Thus, these pro-UK mutants are excellent and safe thrombolytic agents. These advantages allow them to be used in a variety of new methods, devices, and compositions useful for thrombolysis and treating various cardiovascular disorders in clinical situations where administration of other known thrombolytic agents has been too risky or even contraindicated.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2005:36932 USPATFULL
TITLE: Methods, devices, and compositions for lysis of occlusive blood clots while sparing wound sealing clots
INVENTOR(S): Gurewich, Victor, Cambridge, MA, UNITED STATES
Williams, John N., Boston, MA, UNITED STATES

Liu, Jian-Ning, Brighton, MA, UNITED STATES
Sarmientos, Paolo, Lecco, ITALY
Pagani, Massimiliano, Castelli Calepio (Bergamo), ITALY

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2005031607	A1	20050210
APPLICATION INFO.:	US 2004-826826	A1	20040416 (10)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2003-464003P	20030418 (60)
	US 2003-463930P	20030418 (60)
	US 2003-464002P	20030418 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: FISH & RICHARDSON PC, 225 FRANKLIN ST, BOSTON, MA, 02110
NUMBER OF CLAIMS: 19
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 19 Drawing Page(s)
LINE COUNT: 2422
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L3 ANSWER 2 OF 8 USPATFULL on STN
TI Methods of making pro-urokinase mutants
AB Methods are provided for producing non-glycosylated, single-chain and two-chain pro-urokinase (pro-UK) mutants. The methods include cultivating a specific E. coli type B strain that has been transformed with specific plasmids carrying a cDNA sequence that encodes pro-UK mutants and carries specific promoter sequences. Products produced by the methods have medical use for thrombolysis performed while sparing hemostatic clots, e.g., for particular applications such as after a stroke or heart attack.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2005:23319 USPATFULL
TITLE: Methods of making pro-urokinase mutants
INVENTOR(S): Sarmientos, Paolo, Leoco, ITALY
Pagani, Massimiliano, Cividino, ITALY

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2005019863	A1	20050127
APPLICATION INFO.:	US 2004-826598	A1	20040416 (10)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2003-463930P	20030418 (60)
	US 2003-464003P	20030418 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: FISH & RICHARDSON PC, 225 FRANKLIN ST, BOSTON, MA, 02110
NUMBER OF CLAIMS: 24
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 4 Drawing Page(s)
LINE COUNT: 849
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L3 ANSWER 3 OF 8 DGENE COPYRIGHT 2005 The Thomson Corp on STN
TI Use of pro-urokinase (**pro-UK**) **mutant** in
clearing a lumen of blood clots for treating a person with symptoms of

stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery.

AN ADU26339 DNA DGENE

AB The invention relates to the use of pro-urokinase mutants in various methods and devices. It also provides the compositions for therapeutic thrombolysis without inducing haemorrhage. The pro-UK mutants are excellent and safe thrombolytic agents. The invention is based on the discovery of certain mutant forms of pro-urokinase mutants (for example, mutant **M5**). These mutants are plasminogen activators and spare good fibrin clots. The mutants have lower intrinsic activity and are more stable in plasma compared to native pro-UK. Native pro-UK is a protein having 411 amino acids with several different domains including a flexible loop at the amino acid locations 297-313. The pro-UK mutants are shown to have significant advantages over pro-UK and other thrombolytic agents. These advantages allow them to be used in a variety of new methods and devices useful for thrombolysis and treating various cardiovascular disorders. One example of a pro-UK flexible loop mutant is **M5** which has the complete amino acid sequence of the wild type pro-UK except for one amino acid alteration (K300H). The pro-UK mutants dissolve occlusive clots more effectively than native pro-UK. These mutants can be synthesised using site directed mutagenesis (oligonucleotide directed mutagenesis). Oligonucleotide directed mutagenesis is accomplished by synthesising an oligonucleotide primer whose sequence contains the mutation of interest, hybridising the primer to a template containing the native sequence and extending it. The new methods can be used to produce pro-UK mutants on a commercial scale. The high level of purified pro-UK mutants can be obtained by properly selecting different variables (such as a specific promoter sequence, expression plasmid, fermentation, and protein purification technique). The pro-UK mutants of the invention can be stored over time and can directly administered to patients. It can also be combined with other drugs to form compositions which can be administered to the patient in one solution. The **M5** can be used for accurate diagnosis and proper treatment for acute myocardial infarction (myocardial ischaemia), angina pectoris, stroke, diabetes (diabetic myocardium). It can also be used for clearing of a dialysis catheter. The thrombolytic agents are not generally prescribed to the patients over the age of 75 because of higher rates of haemorrhagic complications. The pro- UK flexible loop mutants (**M5**) are beneficial for older patients. The presented nucleotide sequence is the primer 4 which was used to to mutate (K300H) human pro-UK (urokinase).

ACCESSION NUMBER: ADU26339 DNA DGENE
TITLE: Use of pro-urokinase (**pro-UK**) **mutant** in clearing a lumen of blood clots for treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery.
INVENTOR: Gurewich V; Williams J N; Liu J; Sarmientos P; Pagani M
PATENT ASSIGNEE: (THRO-N)THROMBOLYTIC SCI INC.
PATENT INFO: WO 2004093797 A2 20041104 90
APPLICATION INFO: WO 2004-US11840 20040416
PRIORITY INFO: US 2003-463930P 20030418
US 2003-464002P 20030418
US 2003-464003P 20030418
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: 2004-775860 [76]
DESCRIPTION: Primer 4 used to mutate human pro-urokinase (K300H).

L3 ANSWER 4 OF 8 DGENE COPYRIGHT 2005 The Thomson Corp on STN

TI Use of pro-urokinase (**pro-UK**) **mutant** in clearing a lumen of blood clots for treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a

patient before, during or after surgery.

AN ADU26337 DNA DGENE

AB The invention relates to the use of pro-urokinase mutants in various methods and devices. It also provides the compositions for therapeutic thrombolysis without inducing haemorrhage. The pro-UK mutants are excellent and safe thrombolytic agents. The invention is based on the discovery of certain mutant forms of pro-urokinase mutants (for example, mutant **M5**). These mutants are plasminogen activators and spare good fibrin clots. The mutants have lower intrinsic activity and are more stable in plasma compared to native pro-UK. Native pro-UK is a protein having 411 amino acids with several different domains including a flexible loop at the amino acid locations 297-313. The pro-UK mutants are shown to have significant advantages over pro-UK and other thrombolytic agents. These advantages allow them to be used in a variety of new methods and devices useful for thrombolysis and treating various cardiovascular disorders. One example of a pro-UK flexible loop mutant is **M5** which has the complete amino acid sequence of the wild type pro-UK except for one amino acid alteration (K300H). The pro-UK mutants dissolve occlusive clots more effectively than native pro-UK. These mutants can be synthesised using site directed mutagenesis (oligonucleotide directed mutagenesis). Oligonucleotide directed mutagenesis is accomplished by synthesising an oligonucleotide primer whose sequence contains the mutation of interest, hybridising the primer to a template containing the native sequence and extending it. The new methods can be used to produce pro-UK mutants on a commercial scale. The high level of purified pro-UK mutants can be obtained by properly selecting different variables (such as a specific promoter sequence, expression plasmid, fermentation, and protein purification technique). The pro-UK mutants of the invention can be stored over time and can directly administered to patients. It can also be combined with other drugs to form compositions which can be administered to the patient in one solution. The **M5** can be used for accurate diagnosis and proper treatment for acute myocardial infarction (myocardial ischaemia), angina pectoris, stroke, diabetes (diabetic myocardium). It can also be used for clearing of a dialysis catheter. The thrombolytic agents are not generally prescribed to the patients over the age of 75 because of higher rates of haemorrhagic complications. The pro- UK flexible loop mutants (**M5**) are beneficial for older patients. The presented nucleotide sequence is the primer 2 which was used to incorporate restriction sites (Nde1 and SacI) in human pro-UK cDNA.

ACCESSION NUMBER: ADU26337 DNA DGENE

TITLE: Use of pro-urokinase (**pro-UK**) **mutant** in clearing a lumen of blood clots for treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery.

INVENTOR: Gurewich V; Williams J N; Liu J; Sarmientos P; Pagani M

PATENT ASSIGNEE: (THRO-N)THROMBOLYTIC SCI INC.

PATENT INFO: WO 2004093797 A2 20041104 90

APPLICATION INFO: WO 2004-US11840 20040416

PRIORITY INFO: US 2003-463930P 20030418
US 2003-464002P 20030418
US 2003-464003P 20030418

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: 2004-775860 [76]

DESCRIPTION: Primer 2 used to incorporate restriction sites in human pro-UK.

L3 ANSWER 5 OF 8 DGENE COPYRIGHT 2005 The Thomson Corp on STN

TI Use of pro-urokinase (**pro-UK**) **mutant** in clearing a lumen of blood clots for treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a

patient before, during or after surgery.

AN ADU26338 DNA DGENE

AB The invention relates to the use of pro-urokinase mutants in various methods and devices. It also provides the compositions for therapeutic thrombolysis without inducing haemorrhage. The pro-UK mutants are excellent and safe thrombolytic agents. The invention is based on the discovery of certain mutant forms of pro-urokinase mutants (for example, mutant **M5**). These mutants are plasminogen activators and spare good fibrin clots. The mutants have lower intrinsic activity and are more stable in plasma compared to native pro-UK. Native pro-UK is a protein having 411 amino acids with several different domains including a flexible loop at the amino acid locations 297-313. The pro-UK mutants are shown to have significant advantages over pro-UK and other thrombolytic agents. These advantages allow them to be used in a variety of new methods and devices useful for thrombolysis and treating various cardiovascular disorders. One example of a pro-UK flexible loop mutant is **M5** which has the complete amino acid sequence of the wild type pro-UK except for one amino acid alteration (K300H). The pro-UK mutants dissolve occlusive clots more effectively than native pro-UK. These mutants can be synthesised using site directed mutagenesis (oligonucleotide directed mutagenesis). Oligonucleotide directed mutagenesis is accomplished by synthesising an oligonucleotide primer whose sequence contains the mutation of interest, hybridising the primer to a template containing the native sequence and extending it. The new methods can be used to produce pro-UK mutants on a commercial scale. The high level of purified pro-UK mutants can be obtained by properly selecting different variables (such as a specific promoter sequence, expression plasmid, fermentation, and protein purification technique). The pro-UK mutants of the invention can be stored over time and can directly administered to patients. It can also be combined with other drugs to form compositions which can be administered to the patient in one solution. The **M5** can be used for accurate diagnosis and proper treatment for acute myocardial infarction (myocardial ischaemia), angina pectoris, stroke, diabetes (diabetic myocardium). It can also be used for clearing of a dialysis catheter. The thrombolytic agents are not generally prescribed to the patients over the age of 75 because of higher rates of haemorrhagic complications. The pro- UK flexible loop mutants (**M5**) are beneficial for older patients. The presented nucleotide sequence is the primer 3 which was used to to mutate (K300H) human pro-UK (urokinase).

ACCESSION NUMBER: ADU26338 DNA DGENE

TITLE: Use of pro-urokinase (**pro-UK**) **mutant** in clearing a lumen of blood clots for treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery.

INVENTOR: Gurewich V; Williams J N; Liu J; Sarmientos P; Pagani M

PATENT ASSIGNEE: (THRO-N)THROMBOLYTIC SCI INC.

PATENT INFO: WO 2004093797 A2 20041104 90

APPLICATION INFO: WO 2004-US11840 20040416

PRIORITY INFO: US 2003-463930P 20030418
US 2003-464002P 20030418
US 2003-464003P 20030418

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: 2004-775860 [76]

DESCRIPTION: Primer 3 used to mutate human pro-urokinase (K300H).

L3 ANSWER 6 OF 8 DGENE COPYRIGHT 2005 The Thomson Corp on STN

TI Use of pro-urokinase (**pro-UK**) **mutant** in clearing a lumen of blood clots for treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery.

AN ADU26336 DNA DGENE

AB The invention relates to the use of pro-urokinase mutants in various methods and devices. It also provides the compositions for therapeutic thrombolysis without inducing haemorrhage. The pro-UK mutants are excellent and safe thrombolytic agents. The invention is based on the discovery of certain mutant forms of pro-urokinase mutants (for example, mutant **M5**). These mutants are plasminogen activators and spare good fibrin clots. The mutants have lower intrinsic activity and are more stable in plasma compared to native pro-UK. Native pro-UK is a protein having 411 amino acids with several different domains including a flexible loop at the amino acid locations 297-313. The pro-UK mutants are shown to have significant advantages over pro-UK and other thrombolytic agents. These advantages allow them to be used in a variety of new methods and devices useful for thrombolysis and treating various cardiovascular disorders. One example of a pro-UK flexible loop mutant is **M5** which has the complete amino acid sequence of the wild type pro-UK except for one amino acid alteration (K300H). The pro-UK mutants dissolve occlusive clots more effectively than native pro-UK. These mutants can be synthesised using site directed mutagenesis (oligonucleotide directed mutagenesis). Oligonucleotide directed mutagenesis is accomplished by synthesising an oligonucleotide primer whose sequence contains the mutation of interest, hybridising the primer to a template containing the native sequence and extending it. The new methods can be used to produce pro-UK mutants on a commercial scale. The high level of purified pro-UK mutants can be obtained by properly selecting different variables (such as a specific promoter sequence, expression plasmid, fermentation, and protein purification technique). The pro-UK mutants of the invention can be stored over time and can directly administered to patients. It can also be combined with other drugs to form compositions which can be administered to the patient in one solution. The **M5** can be used for accurate diagnosis and proper treatment for acute myocardial infarction (myocardial ischaemia), angina pectoris, stroke, diabetes (diabetic myocardium). It can also be used for clearing of a dialysis catheter. The thrombolytic agents are not generally prescribed to the patients over the age of 75 because of higher rates of haemorrhagic complications. The pro- UK flexible loop mutants (**M5**) are beneficial for older patients. The presented nucleotide sequence is the primer 1 which was used to incorporate restriction sites (Nde1 and SacI) in human pro-UK cDNA.

ACCESSION NUMBER: ADU26336 DNA DGENE

TITLE: Use of pro-urokinase (**pro-UK**) **mutant** in clearing a lumen of blood clots for treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery.

INVENTOR: Gurewich V; Williams J N; Liu J; Sarmientos P; Pagani M

PATENT ASSIGNEE: (THRO-N)THROMBOLYTIC SCI INC.

PATENT INFO: WO 2004093797 A2 20041104 90

APPLICATION INFO: WO 2004-US11840 20040416

PRIORITY INFO: US 2003-463930P 20030418
US 2003-464002P 20030418
US 2003-464003P 20030418

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: 2004-775860 [76]

DESCRIPTION: Primer 1 used to incorporate restriction sites in human pro-UK cDNA.

L3 ANSWER 7 OF 8 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN

TI Use of pro-urokinase (**pro-UK**) **mutant** in clearing a lumen of blood clots for treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery.

AN 2004-775860 [76] WPIDS
AB WO2004093797 A UPAB: 20041125
NOVELTY - A pro-urokinase (**pro-UK**) **mutant** is useful in treating a person with symptoms of stroke or heart attack which comprises determining that the person potentially has had a stroke or heart attack based on observing one or more symptoms of stroke or heart attack and administering to the person a composition comprising an amount of the **pro-UK mutant** effective to lyse any potential blood clot causing the symptoms of stroke or heart attack, is new.

DETAILED DESCRIPTION - A pro-urokinase (**pro-UK**) **mutant** is useful in treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery. INDEPENDENT CLAIMS are also included for:

(1) an intravascular expandable catheter for delivering to a vascular site in a patient an activated, two-chain pro-urokinase (tcpro-UK) mutant;

(2) an intravascular device for delivering to a vascular site in a patient an activated, two-chain pro-urokinase (tcpro-UK) mutant comprising a body and a carrier layer arranged on a surface of the body, where the carrier layer comprises a sustained release agent that slowly releases over time an amount of a tcpro-UK mutant effective to lyse thrombi or emboli in contact with the body;

(3) a method of preparing a pro-urokinase (**pro-UK**) **mutant** polypeptide;

(4) a composition comprising an isolated, single-chain pro-urokinase (**pro-UK mutant** polypeptide, where at least 96% of the protein in the composition is the single-chain **pro-UK mutant** polypeptide;

(5) a purified culture of E. coli type B strain bacteria BL21/DE3 RIL, where bacteria in the culture comprise an expression plasmid encoding a pro-urokinase flexible loop mutant polypeptide; and

(6) preparing a pro-urokinase (**pro-UK**) **mutant** polypeptide.

ACTIVITY - Thrombolytic; Cardiant; Cerebroprotective; Vasotropic. No biological data given.

MECHANISM OF ACTION - Gene therapy.

USE - The pro-urokinase (**pro-UK**) **mutant** is useful in clearing a lumen of blood clots or for lysing occlusive thrombi and emboli for treating a person with symptoms of stroke or heart attack in a patient before, during or after surgery. The composition comprising an aliquot of 20 to 40 mg of a **pro-UK mutant**, packaged with directions is useful in administering as a bolus or by infusion to a patient exhibiting symptoms of a stroke or a heart attack. (All claimed.)

Dwg.0/14

ACCESSION NUMBER: 2004-775860 [76] WPIDS
DOC. NO. CPI: C2004-271684
TITLE: Use of pro-urokinase (**pro-UK**) **mutant** in clearing a lumen of blood clots for treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery.
DERWENT CLASS: B04 D16 P34
INVENTOR(S): GUREWICH, V; LIU, J; PAGANI, M; SARMIENTOS, P; WILLIAMS, J N
PATENT ASSIGNEE(S): (VLII-N) VLI INT LTD UK; (PAGA-I) PAGANI M; (SARM-I) SARMIENTOS P; (GURE-I) GUREWICH V; (LIUJ-I) LIU J; (WILL-I) WILLIAMS J N; (THRO-N) THROMBOLYTIC SCI INC
COUNTRY COUNT: 108
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG

WO 2004093797 A2 20041104 (200476)* EN 90
RW: AT BE BG BW CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE
LS LU MC MW MZ NL OA PL PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH CN CO CR CU CZ DE
DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG
KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NA NI NO NZ
OM PG PH PL PT RO RU SC SD SE SG SK SL SY TJ TM TN TR TT TZ UA UG
US UZ VC VN YU ZA ZM ZW
CA 2426115 A1 20041018 (200476) EN
US 2005019863 A1 20050127 (200509)
US 2005031607 A1 20050210 (200512)

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
WO 2004093797	A2		WO 2004-US11840	20040416
CA 2426115	A1		CA 2003-2426115	20030422
US 2005019863	A1	Provisional	US 2003-463930P	20030418
		Provisional	US 2003-464003P	20030418
			US 2004-826598	20040416
US 2005031607	A1	Provisional	US 2003-463930P	20030418
		Provisional	US 2003-464002P	20030418
		Provisional	US 2003-464003P	20030418
			US 2004-826826	20040416

PRIORITY APPLN. INFO: US 2003-464003P 20030418; US
2003-463930P 20030418; US
2003-464002P 20030418; US
2004-826598 20040416; US
2004-826826 20040416

L3 ANSWER 8 OF 8 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
TI Use of pro-urokinase (**pro-UK**) **mutant** in
clearing a lumen of blood clots for treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery;
recombinant protein production via plasmid expression in host cell for use in disease therapy and gene therapy
AN 2004-26514 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - A pro-urokinase (**pro-UK**) **mutant**
is useful in treating a person with symptoms of stroke or heart attack which comprises determining that the person potentially has had a stroke or heart attack based on observing one or more symptoms of stroke or heart attack and administering to the person a composition comprising an amount of the **pro-UK mutant** effective to
lyse any potential blood clot causing the symptoms of stroke or heart attack, is new.
DETAILED DESCRIPTION - A pro-urokinase (**pro-UK**)
mutant is useful in treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery. INDEPENDENT CLAIMS are also included for: (1) an intravascular expandable catheter for delivering to a vascular site in a patient an activated, two-chain pro-urokinase (tcpro-UK) mutant; (2) an intravascular device for delivering to a vascular site in a patient an activated, two-chain pro-urokinase (tcpro-UK) mutant comprising a body and a carrier layer arranged on a surface of the body, where the carrier layer comprises a sustained release agent that slowly releases over time an amount of a tcpro-UK mutant effective to lyse thrombi or emboli in contact with the body; (3) a method of preparing a pro-urokinase (**pro-UK**)

mutant polypeptide; (4) a composition comprising an isolated, single-chain pro-urokinase (**pro-UK mutant** polypeptide, where at least 96% of the protein in the composition is the single-chain **pro-UK mutant** polypeptide; (5) a purified culture of E. coli type B strain bacteria BL21/DE3 RIL, where bacteria in the culture comprise an expression plasmid encoding a pro-urokinase flexible loop mutant polypeptide; and (6) preparing a pro-urokinase (**pro-UK**) **mutant** polypeptide.

BIOTECHNOLOGY - Preferred Mutant: The new pro-urokinase (**pro-UK**) **mutant** is an activated, two-chain pro-urokinase (tcpro-UK) mutant for clearing a lumen of blood clots, which comprises obtaining a lumen that contains or may contain a blood clot and flowing through the lumen a solution comprising an activated, tcpro-UK mutant for a time sufficient for any blood clots to be dissolved. The solution comprises a concentration of tcpro-UK mutant of 0.05-0.2 mg. The lumen is in a catheter, blood pump or artificial organ. The tcproUK mutant is a low molecular weight tcpro-UK mutant. The new pro-urokinase (**pro-UK**) **mutant** is useful in treating a person with symptoms of stroke or heart attack which comprises determining that the person potentially has had a stroke or heart attack based on observing one or more symptoms of stroke or heart attack and administering to the person a composition comprising an amount of the **pro-UK mutant** effective to lyse any potential blood clot causing the symptoms of stroke or heart attack. The pro-urokinase (**pro-UK**) **mutant** is useful in lysing occlusive thrombi and emboli in a patient before, during, or after surgery, which comprises administering to the patient within 5 hours before surgery, during surgery, or within 24 hours after surgery, a composition comprising the **pro-UK mutant** effective to preferentially lyse any potential occlusive thrombus or embolus compared to hemostatic fibrin in wound sealing clots. The **pro-UK mutant** comprises a pro-UK flexible loop mutant. The **pro-UK mutant** comprises the mutation Lys300 to His. The composition is administered more than 3 hours after the onset of symptoms. A bolus of the composition comprising 20-50 mg of the **pro-UK mutant** is administered. The method further comprises obtaining a medical confirmation of an occlusive thrombus in the brain and administering an infusion of the composition at a **pro-UK mutant** dosage of dose of 120-200 mg/hour (intravenous) or 50-100 mg/hour (intra-arterial). The composition is administered within 90 minutes of the onset of symptoms. The pro-urokinase (**pro-UK**) **mutant** is useful in lysing occlusive thrombi and emboli in a patient before, during, or after surgery, which comprises administering to the patient within 5 hours before surgery, during surgery, or within 24 hours after surgery, a composition comprising the **pro-UK mutant** effective to preferentially lyse any potential occlusive thrombus or embolus compared to hemostatic fibrin in wound sealing clots. The composition is administered by infusion within three hours before, during or after surgery. The composition is administered by infusion at a **pro-UK mutant** dosage of 50 - 200 ml/hour.

Preferred Catheter: The intravascular expandable catheter for delivering to a vascular site in a patient an activated, two-chain pro-urokinase (tcpro-UK) mutant comprises: (1) a catheter body having proximal and distal ends; (2) an expandable portion arranged at the distal end of the catheter body; and (3) a carrier layer arranged on a surface of the expandable portion, where the carrier layer comprises an amount of a tcpro-UK mutant effective to lyse thrombi or emboli in contact with the expandable portion. The carrier layer is a hydrogel selected to quickly release effective amounts of the tcpro-UK mutant upon contact with a thrombus or embolus. The amount of the tcpro-UK mutant comprises 0.1-0.5 mg. The carrier layer comprises a lumen containing the tcpro-UK mutant and one or more apertures that are pressed against a thrombus or embolus

to allow the thrombus or embolus to protrude into the one or more apertures, thereby contacting the tcpro-UK mutant. The carrier layer comprises activated, two-chain **pro-UK mutant M5**. Preferred Device: The device is a stent or suture. Preferred Composition: The composition comprises an isolated, single-chain pro-urokinase (**pro-UK**) **mutant** polypeptide, where at least 98% of the protein in the composition is the single-chain **pro-UK mutant** polypeptide, and an acidic excipient. Preferred Method: Preparing a pro-urokinase (**pro-UK**) **mutant** polypeptide comprises: (1) obtaining a nucleic acid molecule that encodes a **pro-UK mutant** polypeptide; (2) inserting the nucleic acid molecule into a pET29a expression plasmid comprising a phage T7 promoter and Shine-Dalgarno sequence; (3) transforming E. coli type B strain bacteria BL21/DE3 RIL with the expression plasmid; (4) culturing the transformed bacteria for a time and under conditions sufficient to enable the bacteria to express **pro-UK mutant** polypeptide; and (5) isolating the **pro-UK mutant** polypeptide from the transformed bacteria. The **pro-UK mutant** is non-glycosylated and has a molecular weight of about 45,000 daltons. The culturing comprises a two-stage fermentation. The first stage of fermentation comprises adding to a flask a cell culture diluted in sterile EC1 medium and growing the culture at about 34 to 37 degrees C for at least about 10 hours with agitation to form a seed culture, where the cell culture comprises a glycerol suspension of an LB culture of the transformed bacteria and containing a sufficient amount of kanamycin. The second stage of fermentation comprises: (1) adding the seed culture to a fermenter; (2) maintaining the pH in the fermenter at about 6.8 to 7.2; (3) maintaining the dissolved oxygen concentration in the culture medium at about 35 to 45% of air saturation; (4) maintaining the temperature of fermentation at about 34 to 37 degrees C; and (5) adding to the fermentor a nutrient feeding solution comprising one or more sugars when all glucose initially present in the fermentor at step (1) is consumed following the equation V = Vo e18t. V = is volume of feeding solution added (ml/h); Vo = is 1/100 of the starting fermentation medium (ml); and t = is time of fermentation after the start of the feeding phase (hours). The expression plasmid containing the nucleic acid molecule is pET29aUKM5. The method further comprises preparing two-chain **pro-UK mutant** by passing the **pro-UK mutant** over plasmin bound to a substrate. The substrate is an agarose-based gel filtration medium. The method further comprises combining the isolated **pro-UK mutant** polypeptide with an acidic excipient. Preparing a pro-urokinase (**pro-UK**) **mutant** polypeptide comprises: (1) obtaining a transformed bacteria, where the bacteria is an E. coli type B strain bacteria BL21/DE3 RIL transformed with a pET29a expression plasmid comprising a phage T7 promoter, a Shine-Dalgarno sequence, and a nucleic acid molecule that encodes a **pro-UK mutant** polypeptide; (2) culturing the transformed bacteria for a time and under conditions sufficient to enable the bacteria to express **pro-UK mutant** polypeptide; and (3) isolating the **pro-UK mutant** polypeptide from the transformed bacteria.

ACTIVITY - Thrombolytic; Cardiant; Cerebroprotective; Vasotropic. No biological data given.

MECHANISM OF ACTION - Gene therapy.

USE - The pro-urokinase (**pro-UK**) **mutant** is useful in clearing a lumen of blood clots or for lysing occlusive thrombi and emboli for treating a person with symptoms of stroke or heart attack in a patient before, during or after surgery. The composition comprising an aliquot of 20 to 40 mg of a **pro-UK mutant**, packaged with directions is useful in administering as a bolus or by infusion to a patient exhibiting symptoms of a stroke or a

heart attack. (All claimed.)
ADMINISTRATION - The composition is administered via intravenous route. No biological data given.
EXAMPLE - No relevant examples given. (90 pages)

ACCESSION NUMBER: 2004-26514 BIOTECHDS
TITLE: Use of pro-urokinase (**pro-UK**) **mutant** in clearing a lumen of blood clots for treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery;
recombinant protein production via plasmid expression in host cell for use in disease therapy and gene therapy
AUTHOR: GUREWICH V; WILLIAMS J N; LIU J; SARMIENTOS P; PAGANI M
PATENT ASSIGNEE: THROMBOLYTIC SCI INC
PATENT INFO: WO 2004093797 4 Nov 2004
APPLICATION INFO: WO 2004-US11840 16 Apr 2004
PRIORITY INFO: US 2003-464003 18 Apr 2003; US 2003-463930 18 Apr 2003
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-775860 [76]

=> s (E coli type B strain)
5 FILES SEARCHED...
L4 6 (E COLI TYPE B STRAIN)

=> d l4 ti abs ibib tot

L4 ANSWER 1 OF 6 USPATFULL on STN
TI Methods, devices, and compositions for lysis of occlusive blood clots while sparing wound sealing clots
AB It has now been discovered that certain mutant forms of pro-urokinase ("pro-UK"), such as so-called pro-UK mutant "M5" (Lys.sup.300→His), perform in the manner of pro-UK in lysing "bad" blood clots (those clots that occlude blood vessels), while sparing hemostatic fibrin in the so-called "good" blood clots (those clots that seal wounds, e.g., after surgery or other tissue injury). Thus, these pro-UK mutants are excellent and safe thrombolytic agents. These advantages allow them to be used in a variety of new methods, devices, and compositions useful for thrombolysis and treating various cardiovascular disorders in clinical situations where administration of other known thrombolytic agents has been too risky or even contraindicated.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2005:36932 USPATFULL
TITLE: Methods, devices, and compositions for lysis of occlusive blood clots while sparing wound sealing clots
INVENTOR(S): Gurewich, Victor, Cambridge, MA, UNITED STATES
Williams, John N., Boston, MA, UNITED STATES
Liu, Jian-Ning, Brighton, MA, UNITED STATES
Sarmientos, Paolo, Lecco, ITALY
Pagani, Massimiliano, Castelli Calepio (Bergamo), ITALY

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2005031607	A1	20050210	
APPLICATION INFO.:	US 2004-826826	A1	20040416	(10)

	NUMBER	DATE	
PRIORITY INFORMATION:	US 2003-464003P	20030418	(60)
	US 2003-463930P	20030418	(60)

	US 2003-464002P 20030418 (60)
DOCUMENT TYPE:	Utility
FILE SEGMENT:	APPLICATION
LEGAL REPRESENTATIVE:	FISH & RICHARDSON PC, 225 FRANKLIN ST, BOSTON, MA, 02110
NUMBER OF CLAIMS:	19
EXEMPLARY CLAIM:	1
NUMBER OF DRAWINGS:	19 Drawing Page(s)
LINE COUNT:	2422

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 2 OF 6 USPATFULL on STN

TI Methods of making pro-urokinase mutants

AB Methods are provided for producing non-glycosylated, single-chain and two-chain pro-urokinase (pro-UK) mutants. The methods include cultivating a specific **E. coli type B strain** that has been transformed with specific plasmids carrying a cDNA sequence that encodes pro-UK mutants and carries specific promoter sequences. Products produced by the methods have medical use for thrombolysis performed while sparing hemostatic clots, e.g., for particular applications such as after a stroke or heart attack.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2005:23319 USPATFULL
TITLE:	Methods of making pro-urokinase mutants
INVENTOR(S):	Sarmientos, Paolo, Leoco, ITALY Pagani, Massimiliano, Cividino, ITALY

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2005019863	A1	20050127	
APPLICATION INFO.:	US 2004-826598	A1	20040416	(10)

	NUMBER	DATE	
PRIORITY INFORMATION:	US 2003-463930P	20030418	(60)
	US 2003-464003P	20030418	(60)
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	APPLICATION		
LEGAL REPRESENTATIVE:	FISH & RICHARDSON PC, 225 FRANKLIN ST, BOSTON, MA, 02110		
NUMBER OF CLAIMS:	24		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	4 Drawing Page(s)		
LINE COUNT:	849		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 3 OF 6 USPATFULL on STN

TI Deletion mutant of basic fibroblast growth factor and production thereof

AB The present invention relates to the production, by recombinant DNA techniques, of derivatives of basic fibroblast growth factor (bFGF). These derivatives of bFGF can act as antagonists and/or superagonists of the wild type molecule in the angiogenic process. These derivatives, as well as wild type bFGF, may be prepared by the use of strains of E. coli which have been transformed with plasmids carrying nucleotide sequence coding for human and bovine bFGF and their derivatives.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	94:86319 USPATFULL
TITLE:	Deletion mutant of basic fibroblast growth factor and production thereof
INVENTOR(S):	Bergonzoni, Laura, Milan, Italy

Isacchi, Antonella, Milan, Italy
Sarmientos, Paolo, Milan, Italy
Cauet, Gilles, Buccinasco, Italy

PATENT ASSIGNEE(S): Farmitalia Carlo Erba S.R.L., Milan, Italy (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5352589		19941004
APPLICATION INFO.:	US 1993-71046		19930602 (8)

RELATED APPLN. INFO.: Continuation of Ser. No. US 1992-863549, filed on 6 Apr 1992, now abandoned which is a continuation of Ser. No. US 1990-466441, filed on 16 Jul 1990, now abandoned

	NUMBER	DATE
PRIORITY INFORMATION:	GB 1988-217955	19880916

DOCUMENT TYPE: Utility
FILE SEGMENT: Granted
PRIMARY EXAMINER: Hill, Jr., Robert J.
ASSISTANT EXAMINER: Allen, Marianne Porta
LEGAL REPRESENTATIVE: Oblon, Spivak, McClelland, Maier & Neustadt
NUMBER OF CLAIMS: 3
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 15 Drawing Figure(s); 9 Drawing Page(s)
LINE COUNT: 665
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L4 ANSWER 4 OF 6 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN

TI Use of pro-urokinase (pro-UK) mutant in clearing a lumen of blood clots for treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery.

AN 2004-775860 [76] WPIDS

AB WO2004093797 A UPAB: 20041125

NOVELTY - A pro-urokinase (pro-UK) mutant is useful in treating a person with symptoms of stroke or heart attack which comprises determining that the person potentially has had a stroke or heart attack based on observing one or more symptoms of stroke or heart attack and administering to the person a composition comprising an amount of the pro-UK mutant effective to lyse any potential blood clot causing the symptoms of stroke or heart attack, is new.

DETAILED DESCRIPTION - A pro-urokinase (pro-UK) mutant is useful in treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery. INDEPENDENT CLAIMS are also included for:

(1) an intravascular expandable catheter for delivering to a vascular site in a patient an activated, two-chain pro-urokinase (tcpro-UK) mutant;

(2) an intravascular device for delivering to a vascular site in a patient an activated, two-chain pro-urokinase (tcpro-UK) mutant comprising a body and a carrier layer arranged on a surface of the body, where the carrier layer comprises a sustained release agent that slowly releases over time an amount of a tcpro-UK mutant effective to lyse thrombi or emboli in contact with the body;

(3) a method of preparing a pro-urokinase (pro-UK) mutant polypeptide;

(4) a composition comprising an isolated, single-chain pro-urokinase (pro-UK mutant polypeptide, where at least 96% of the protein in the composition is the single-chain pro-UK mutant polypeptide;

(5) a purified culture of **E. coli type B strain** bacteria BL21/DE3 RIL, where bacteria in the culture comprise an expression plasmid encoding a pro-urokinase flexible loop mutant polypeptide; and

(6) preparing a pro-urokinase (pro-UK) mutant polypeptide.

ACTIVITY - Thrombolytic; Cardiant; Cerebroprotective; Vasotropic.
No biological data given.
MECHANISM OF ACTION - Gene therapy.
USE - The pro-urokinase (pro-UK) mutant is useful in clearing a lumen of blood clots or for lysing occlusive thrombi and emboli for treating a person with symptoms of stroke or heart attack in a patient before, during or after surgery. The composition comprising an aliquot of 20 to 40 mg of a pro-UK mutant, packaged with directions is useful in administering as a bolus or by infusion to a patient exhibiting symptoms of a stroke or a heart attack. (All claimed.)
Dwg.0/14

ACCESSION NUMBER: 2004-775860 [76] WPIDS
DOC. NO. CPI: C2004-271684
TITLE: Use of pro-urokinase (pro-UK) mutant in clearing a lumen of blood clots for treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery.
DERWENT CLASS: B04 D16 P34
INVENTOR(S): GUREWICH, V; LIU, J; PAGANI, M; SARMIENTOS, P; WILLIAMS, J N
PATENT ASSIGNEE(S): (VLII-N) VLI INT LTD UK; (PAGA-I) PAGANI M; (SARM-I) SARMIENTOS P; (GURE-I) GUREWICH V; (LIUJ-I) LIU J; (WILL-I) WILLIAMS J N; (THRO-N) THROMBOLYTIC SCI INC
COUNTRY COUNT: 108
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2004093797	A2	20041104	(200476)*	EN	90
RW: AT BE BG BW CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE LS LU MC MW MZ NL OA PL PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW					
W: AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NA NI NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL SY TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW					
CA 2426115	A1	20041018	(200476)	EN	
US 2005019863	A1	20050127	(200509)		
US 2005031607	A1	20050210	(200512)		

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
WO 2004093797	A2		WO 2004-US11840	20040416
CA 2426115	A1		CA 2003-2426115	20030422
US 2005019863	A1	Provisional	US 2003-463930P	20030418
		Provisional	US 2003-464003P	20030418
			US 2004-826598	20040416
US 2005031607	A1	Provisional	US 2003-463930P	20030418
		Provisional	US 2003-464002P	20030418
		Provisional	US 2003-464003P	20030418
			US 2004-826826	20040416

PRIORITY APPLN. INFO: US 2003-464003P 20030418; US 2003-463930P 20030418; US 2003-464002P 20030418; US 2004-826598 20040416; US 2004-826826 20040416

L4 ANSWER 5 OF 6 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
TI Use of pro-urokinase (pro-UK) mutant in clearing a lumen of blood clots

for treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery;

recombinant protein production via plasmid expression in host cell for use in disease therapy and gene therapy

AN 2004-26514 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - A pro-urokinase (pro-UK) mutant is useful in treating a person with symptoms of stroke or heart attack which comprises determining that the person potentially has had a stroke or heart attack based on observing one or more symptoms of stroke or heart attack and administering to the person a composition comprising an amount of the pro-UK mutant effective to lyse any potential blood clot causing the symptoms of stroke or heart attack, is new.

DETAILED DESCRIPTION - A pro-urokinase (pro-UK) mutant is useful in treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery. INDEPENDENT CLAIMS are also included for: (1) an intravascular expandable catheter for delivering to a vascular site in a patient an activated, two-chain pro-urokinase (tcpro-UK) mutant; (2) an intravascular device for delivering to a vascular site in a patient an activated, two-chain pro-urokinase (tcpro-UK) mutant comprising a body and a carrier layer arranged on a surface of the body, where the carrier layer comprises a sustained release agent that slowly releases over time an amount of a tcpro-UK mutant effective to lyse thrombi or emboli in contact with the body; (3) a method of preparing a pro-urokinase (pro-UK) mutant polypeptide; (4) a composition comprising an isolated, single-chain pro-urokinase (pro-UK mutant polypeptide, where at least 96% of the protein in the composition is the single-chain pro-UK mutant polypeptide; (5) a purified culture of **E. coli type B strain** bacteria BL21/DE3 RIL, where bacteria in the culture comprise an expression plasmid encoding a pro-urokinase flexible loop mutant polypeptide; and (6) preparing a pro-urokinase (pro-UK) mutant polypeptide.

BIOTECHNOLOGY - Preferred Mutant: The new pro-urokinase (pro-UK) mutant is an activated, two-chain pro-urokinase (tcpro-UK) mutant for clearing a lumen of blood clots, which comprises obtaining a lumen that contains or may contain a blood clot and flowing through the lumen a solution comprising an activated, tcpro-UK mutant for a time sufficient for any blood clots to be dissolved. The solution comprises a concentration of tcpro-UK mutant of 0.05-0.2 mg. The lumen is in a catheter, blood pump or artificial organ. The tcproUK mutant is a low molecular weight tcpro-UK mutant. The new pro-urokinase (pro-UK) mutant is useful in treating a person with symptoms of stroke or heart attack which comprises determining that the person potentially has had a stroke or heart attack based on observing one or more symptoms of stroke or heart attack and administering to the person a composition comprising an amount of the pro-UK mutant effective to lyse any potential blood clot causing the symptoms of stroke or heart attack. The pro-urokinase (pro-UK) mutant is useful in lysing occlusive thrombi and emboli in a patient before, during, or after surgery, which comprises administering to the patient within 5 hours before surgery, during surgery, or within 24 hours after surgery, a composition comprising the pro-UK mutant effective to preferentially lyse any potential occlusive thrombus or embolus compared to hemostatic fibrin in wound sealing clots. The pro-UK mutant comprises a pro-UK flexible loop mutant. The pro-UK mutant comprises the mutation Lys300 to His. The composition is administered more than 3 hours after the onset of symptoms. A bolus of the composition comprising 20-50 mg of the pro-UK mutant is administered. The method further comprises obtaining a medical confirmation of an occlusive thrombus in the brain and administering an infusion of the composition at a pro-UK mutant dosage of dose of 120-200 mg/hour (intravenous) or 50-100 mg/hour (intra-arterial). The composition is administered within 90

minutes of the onset of symptoms. The pro-urokinase (pro-UK) mutant is useful in lysing occlusive thrombi and emboli in a patient before, during, or after surgery, which comprises administering to the patient within 5 hours before surgery, during surgery, or within 24 hours after surgery, a composition comprising the pro-UK mutant effective to preferentially lyse any potential occlusive thrombus or embolus compared to hemostatic fibrin in wound sealing clots. The composition is administered by infusion within three hours before, during or after surgery. The composition is administered by infusion at a pro-UK mutant dosage of 50 - 200 ml/hour. Preferred Catheter: The intravascular expandable catheter for delivering to a vascular site in a patient an activated, two-chain pro-urokinase (tcpro-UK) mutant comprises: (1) a catheter body having proximal and distal ends; (2) an expandable portion arranged at the distal end of the catheter body; and (3) a carrier layer arranged on a surface of the expandable portion, where the carrier layer comprises an amount of a tcpro-UK mutant effective to lyse thrombi or emboli in contact with the expandable portion. The carrier layer is a hydrogel selected to quickly release effective amounts of the tcpro-UK mutant upon contact with a thrombus or embolus. The amount of the tcpro-UK mutant comprises 0.1-0.5 mg. The carrier layer comprises a lumen containing the tcpro-UK mutant and one or more apertures that are pressed against a thrombus or embolus to allow the thrombus or embolus to protrude into the one or more apertures, thereby contacting the tcpro-UK mutant. The carrier layer comprises activated, two-chain pro-UK mutant M5. Preferred Device: The device is a stent or suture. Preferred Composition: The composition comprises an isolated, single-chain pro-urokinase (pro-UK) mutant polypeptide, where at least 98% of the protein in the composition is the single-chain pro-UK mutant polypeptide, and an acidic excipient. Preferred Method: Preparing a pro-urokinase (pro-UK) mutant polypeptide comprises: (1) obtaining a nucleic acid molecule that encodes a pro-UK mutant polypeptide; (2) inserting the nucleic acid molecule into a pET29a expression plasmid comprising a phage T7 promoter and Shine-Dalgarno sequence; (3) transforming **E. coli type B strain** bacteria BL21/DE3 RIL with the expression plasmid; (4) culturing the transformed bacteria for a time and under conditions sufficient to enable the bacteria to express pro-UK mutant polypeptide; and (5) isolating the pro-UK mutant polypeptide from the transformed bacteria. The pro-UK mutant is non-glycosylated and has a molecular weight of about 45,000 daltons. The culturing comprises a two-stage fermentation. The first stage of fermentation comprises adding to a flask a cell culture diluted in sterile EC1 medium and growing the culture at about 34 to 37 degrees C for at least about 10 hours with agitation to form a seed culture, where the cell culture comprises a glycerol suspension of an LB culture of the transformed bacteria and containing a sufficient amount of kanamycin. The second stage of fermentation comprises: (1) adding the seed culture to a fermenter; (2) maintaining the pH in the fermenter at about 6.8 to 7.2; (3) maintaining the dissolved oxygen concentration in the culture medium at about 35 to 45% of air saturation; (4) maintaining the temperature of fermentation at about 34 to 37 degrees C; and (5) adding to the fermentor a nutrient feeding solution comprising one or more sugars when all glucose initially present in the fermentor at step (1) is consumed following the equation $V = Vo\ e^{18t}$. V = is volume of feeding solution added (ml/h); Vo = is 1/100 of the starting fermentation medium (ml); and t = is time of fermentation after the start of the feeding phase (hours). The expression plasmid containing the nucleic acid molecule is pET29aUKM5. The method further comprises preparing two-chain pro-UK mutant by passing the pro-UK mutant over plasmin bound to a substrate. The substrate is an agarose-based gel filtration medium. The method further comprises combining the isolated pro-UK mutant polypeptide with an acidic excipient. Preparing a pro-urokinase (pro-UK) mutant polypeptide comprises: (1) obtaining a transformed bacteria, where the bacteria is an **E. coli type B**

strain bacteria BL21/DE3 RIL transformed with a pET29a expression plasmid comprising a phage T7 promoter, a Shine-Dalgarno sequence, and a nucleic acid molecule that encodes a pro-UK mutant polypeptide; (2) culturing the transformed bacteria for a time and under conditions sufficient to enable the bacteria to express pro-UK mutant polypeptide; and (3) isolating the pro-UK mutant polypeptide from the transformed bacteria.

ACTIVITY - Thrombolytic; Cardiant; Cerebroprotective; Vasotropic. No biological data given.

MECHANISM OF ACTION - Gene therapy.

USE - The pro-urokinase (pro-UK) mutant is useful in clearing a lumen of blood clots or for lysing occlusive thrombi and emboli for treating a person with symptoms of stroke or heart attack in a patient before, during or after surgery. The composition comprising an aliquot of 20 to 40 mg of a pro-UK mutant, packaged with directions is useful in administering as a bolus or by infusion to a patient exhibiting symptoms of a stroke or a heart attack. (All claimed.)

ADMINISTRATION - The composition is administered via intravenous route. No biological data given.

EXAMPLE - No relevant examples given. (90 pages)

ACCESSION NUMBER: 2004-26514 BIOTECHDS
TITLE: Use of pro-urokinase (pro-UK) mutant in clearing a lumen of blood clots for treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery;
recombinant protein production via plasmid expression in host cell for use in disease therapy and gene therapy
AUTHOR: GUREWICH V; WILLIAMS J N; LIU J; SARMIENTOS P; PAGANI M
PATENT ASSIGNEE: THROMBOLYTIC SCI INC
PATENT INFO: WO 2004093797 4 Nov 2004
APPLICATION INFO: WO 2004-US11840 16 Apr 2004
PRIORITY INFO: US 2003-464003 18 Apr 2003; US 2003-463930 18 Apr 2003
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-775860 [76]

L4 ANSWER 6 OF 6 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
TI Human basic fibroblast growth factor: production of a clinical grade recombinant protein from Escherichia coli;
recombinant protein isolation, purification, characterization and synthetic gene cloning in vector plasmid pFC81; vulnerary activity
AN 1990-10556 BIOTECHDS
AB Recombinant basic fibroblast growth factor (bFGF) was produced in Escherichia coli. A synthetic gene encoding 155 amino acid residues of human bFGF was inserted into plasmid pFC7 under the control of the tryptophan promoter to form plasmid pFC81. This vector was used to transform an **E. coli type B strain**. Fermentation was performed for 20 hr at 37 deg in 10 l fermentors containing 4 l of culture medium, which lacked tryptophan but contained tetracycline in the medium. Bacterial pellets after fermentation were resuspended in phosphate buffer of pH 7.5 and sonicated. The cell lyzate was centrifuged and the resulting supernatant was subjected to S-Sepharose chromatography. bFGF was eluted using a linear NaCl gradient (0.3-1.0 M). Pooled fractions were subjected to heparin-Sepharose chromatography and bFGF was eluted with a linear NaCl gradient (1.0-3.0 M). The recombinant protein was obtained in a yield of 250 mg/l, had mol.weight 18,000 and cross-reacted in western blotting with anti-bFGF antibodies. The purity of the recombinant protein was 95% by SDS-PAGE and it exhibited vulnerary activity e.g. on rabbit cornea. (12 ref)

ACCESSION NUMBER: 1990-10556 BIOTECHDS
TITLE: Human basic fibroblast growth factor: production of a clinical grade recombinant protein from Escherichia coli;

recombinant protein isolation, purification, characterization and synthetic gene cloning in vector plasmid pFC81; vulnerary activity

AUTHOR: Isacchi A; Caccia P; Gauet G; Bertolero F; Bergonzoni L; *Sarmientos P

CORPORATE SOURCE: Farmitalia

LOCATION: R+D Department, Farmitalia Carlo Erba, Viale Bezzi, 24-20146, Milan, Italy.

SOURCE: Chim.Oggi; (1990) 8, 6, 72-74

DOCUMENT TYPE: Journal

LANGUAGE: English

=> d his

(FILE 'HOME' ENTERED AT 14:24:41 ON 18 NOV 2005)

FILE 'MEDLINE, USPATFULL, DGENE, EMBASE, WPIDS, FSTA, JICST-EPLUS, BIOSIS, BIOTECHDS' ENTERED AT 14:25:14 ON 18 NOV 2005

L1 43 S (PRO-UROKINASE MUTANT OR PRO-UK MUTANT)

L2 0 S L1 AND (PREPARATION METHOD)

L3 8 S L1 AND M5

L4 6 S (E COLI TYPE B STRAIN)

=> s l1 and (lys300 to His)

L5 2 L1 AND (LYS300 TO HIS)

=> d l5 ti abs ibib tot

L5 ANSWER 1 OF 2 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN

TI Use of pro-urokinase (**pro-UK**) **mutant** in clearing a lumen of blood clots for treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery.

AN 2004-775860 [76] WPIDS

AB WO2004093797 A UPAB: 20041125

NOVELTY - A pro-urokinase (**pro-UK**) **mutant** is useful in treating a person with symptoms of stroke or heart attack which comprises determining that the person potentially has had a stroke or heart attack based on observing one or more symptoms of stroke or heart attack and administering to the person a composition comprising an amount of the **pro-UK mutant** effective to lyse any potential blood clot causing the symptoms of stroke or heart attack, is new.

DETAILED DESCRIPTION - A pro-urokinase (**pro-UK**) **mutant** is useful in treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery. INDEPENDENT CLAIMS are also included for:

(1) an intravascular expandable catheter for delivering to a vascular site in a patient an activated, two-chain pro-urokinase (tcpro-UK) mutant;

(2) an intravascular device for delivering to a vascular site in a patient an activated, two-chain pro-urokinase (tcpro-UK) mutant comprising a body and a carrier layer arranged on a surface of the body, where the carrier layer comprises a sustained release agent that slowly releases over time an amount of a tcpro-UK mutant effective to lyse thrombi or emboli in contact with the body;

(3) a method of preparing a pro-urokinase (**pro-UK**) **mutant** polypeptide;

(4) a composition comprising an isolated, single-chain pro-urokinase (**pro-UK mutant** polypeptide, where at least 96% of the protein in the composition is the single-chain **pro-UK mutant** polypeptide;

(5) a purified culture of E. coli type B strain bacteria BL21/DE3

RIL, where bacteria in the culture comprise an expression plasmid encoding a pro-urokinase flexible loop mutant polypeptide; and

(6) preparing a pro-urokinase (**pro-UK**) **mutant** polypeptide.

ACTIVITY - Thrombolytic; Cardiant; Cerebroprotective; Vasotropic.

No biological data given.

MECHANISM OF ACTION - Gene therapy.

USE - The pro-urokinase (**pro-UK**) **mutant** is useful in clearing a lumen of blood clots or for lysing occlusive thrombi and emboli for treating a person with symptoms of stroke or heart attack in a patient before, during or after surgery. The composition comprising an aliquot of 20 to 40 mg of a **pro-UK mutant**, packaged with directions is useful in administering as a bolus or by infusion to a patient exhibiting symptoms of a stroke or a heart attack. (All claimed.)

Dwg.0/14

ACCESSION NUMBER: 2004-775860 [76] WPIDS

DOC. NO. CPI: C2004-271684

TITLE: Use of pro-urokinase (**pro-UK**) **mutant** in clearing a lumen of blood clots for treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery.

DERWENT CLASS: B04 D16 P34

INVENTOR(S): GUREWICH, V; LIU, J; PAGANI, M; SARMIENTOS, P; WILLIAMS, J N

PATENT ASSIGNEE(S): (VLII-N) VLI INT LTD UK; (PAGA-I) PAGANI M; (SARM-I) SARMIENTOS P; (GURE-I) GUREWICH V; (LIUJ-I) LIU J; (WILL-I) WILLIAMS J N; (THRO-N) THROMBOLYTIC SCI INC

COUNTRY COUNT: 108

PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2004093797	A2	20041104	(200476)*	EN	90
RW: AT BE BG BW CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE LS LU MC MW MZ NL OA PL PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW					
W: AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NA NI NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL SY TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW					
CA 2426115	A1	20041018	(200476)	EN	
US 2005019863	A1	20050127	(200509)		
US 2005031607	A1	20050210	(200512)		

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
WO 2004093797	A2		WO 2004-US11840	20040416
CA 2426115	A1		CA 2003-2426115	20030422
US 2005019863	A1	Provisional	US 2003-463930P	20030418
		Provisional	US 2003-464003P	20030418
			US 2004-826598	20040416
US 2005031607	A1	Provisional	US 2003-463930P	20030418
		Provisional	US 2003-464002P	20030418
		Provisional	US 2003-464003P	20030418
			US 2004-826826	20040416

PRIORITY APPLN. INFO: US 2003-464003P 20030418; US 2003-463930P 20030418; US

2003-464002P 20030418; US
2004-826598 20040416; US
2004-826826 20040416

L5 ANSWER 2 OF 2 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
TI Use of pro-urokinase (**pro-UK**) **mutant** in clearing a lumen of blood clots for treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery;
recombinant protein production via plasmid expression in host cell for use in disease therapy and gene therapy
AN 2004-26514 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - A pro-urokinase (**pro-UK**) **mutant** is useful in treating a person with symptoms of stroke or heart attack which comprises determining that the person potentially has had a stroke or heart attack based on observing one or more symptoms of stroke or heart attack and administering to the person a composition comprising an amount of the **pro-UK mutant** effective to lyse any potential blood clot causing the symptoms of stroke or heart attack, is new.

DETAILED DESCRIPTION - A pro-urokinase (**pro-UK**) **mutant** is useful in treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery. INDEPENDENT CLAIMS are also included for: (1) an intravascular expandable catheter for delivering to a vascular site in a patient an activated, two-chain pro-urokinase (tcpro-UK) mutant; (2) an intravascular device for delivering to a vascular site in a patient an activated, two-chain pro-urokinase (tcpro-UK) mutant comprising a body and a carrier layer arranged on a surface of the body, where the carrier layer comprises a sustained release agent that slowly releases over time an amount of a tcpro-UK mutant effective to lyse thrombi or emboli in contact with the body; (3) a method of preparing a pro-urokinase (**pro-UK**) **mutant** polypeptide; (4) a composition comprising an isolated, single-chain pro-urokinase (**pro-UK mutant** polypeptide, where at least 96% of the protein in the composition is the single-chain **pro-UK mutant** polypeptide; (5) a purified culture of E. coli type B strain bacteria BL21/DE3 RIL, where bacteria in the culture comprise an expression plasmid encoding a pro-urokinase flexible loop mutant polypeptide; and (6) preparing a pro-urokinase (**pro-UK**) **mutant** polypeptide.

BIOTECHNOLOGY - Preferred Mutant: The new pro-urokinase (**pro-UK**) **mutant** is an activated, two-chain pro-urokinase (tcpro-UK) mutant for clearing a lumen of blood clots, which comprises obtaining a lumen that contains or may contain a blood clot and flowing through the lumen a solution comprising an activated, tcpro-UK mutant for a time sufficient for any blood clots to be dissolved. The solution comprises a concentration of tcpro-UK mutant of 0.05-0.2 mg. The lumen is in a catheter, blood pump or artificial organ. The tcproUK mutant is a low molecular weight tcpro-UK mutant. The new pro-urokinase (**pro-UK**) **mutant** is useful in treating a person with symptoms of stroke or heart attack which comprises determining that the person potentially has had a stroke or heart attack based on observing one or more symptoms of stroke or heart attack and administering to the person a composition comprising an amount of the **pro-UK mutant** effective to lyse any potential blood clot causing the symptoms of stroke or heart attack. The pro-urokinase (**pro-UK**) **mutant** is useful in lysing occlusive thrombi and emboli in a patient before, during, or after surgery, which comprises administering to the patient within 5 hours before surgery, during surgery, or within 24 hours after surgery, a composition comprising the **pro-UK mutant** effective to preferentially

lyse any potential occlusive thrombus or embolus compared to hemostatic fibrin in wound sealing clots. The **pro-UK mutant** comprises a pro-UK flexible loop mutant. The **pro-UK mutant** comprises the mutation **Lys300** to **His**. The composition is administered more than 3 hours after the onset of symptoms. A bolus of the composition comprising 20-50 mg of the **pro-UK mutant** is administered. The method further comprises obtaining a medical confirmation of an occlusive thrombus in the brain and administering an infusion of the composition at a **pro-UK mutant** dosage of dose of 120-200 mg/hour (intravenous) or 50-100 mg/hour (intra-arterial). The composition is administered within 90 minutes of the onset of symptoms. The pro-urokinase (**pro-UK**) **mutant** is useful in lysing occlusive thrombi and emboli in a patient before, during, or after surgery, which comprises administering to the patient within 5 hours before surgery, during surgery, or within 24 hours after surgery, a composition comprising the **pro-UK mutant** effective to preferentially lyse any potential occlusive thrombus or embolus compared to hemostatic fibrin in wound sealing clots. The composition is administered by infusion within three hours before, during or after surgery. The composition is administered by infusion at a **pro-UK mutant** dosage of 50 - 200 ml/hour.

Preferred Catheter: The intravascular expandable catheter for delivering to a vascular site in a patient an activated, two-chain pro-urokinase (tcpro-UK) mutant comprises: (1) a catheter body having proximal and distal ends; (2) an expandable portion arranged at the distal end of the catheter body; and (3) a carrier layer arranged on a surface of the expandable portion, where the carrier layer comprises an amount of a tcpro-UK mutant effective to lyse thrombi or emboli in contact with the expandable portion. The carrier layer is a hydrogel selected to quickly release effective amounts of the tcpro-UK mutant upon contact with a thrombus or embolus. The amount of the tcpro-UK mutant comprises 0.1-0.5 mg. The carrier layer comprises a lumen containing the tcpro-UK mutant and one or more apertures that are pressed against a thrombus or embolus to allow the thrombus or embolus to protrude into the one or more apertures, thereby contacting the tcpro-UK mutant. The carrier layer comprises activated, two-chain **pro-UK mutant** M5. Preferred Device: The device is a stent or suture. Preferred Composition: The composition comprises an isolated, single-chain pro-urokinase (**pro-UK**) **mutant** polypeptide, where at least 98% of the protein in the composition is the single-chain **pro-UK mutant** polypeptide, and an acidic excipient. Preferred Method: Preparing a pro-urokinase (**pro-UK**) **mutant** polypeptide comprises: (1) obtaining a nucleic acid molecule that encodes a **pro-UK mutant** polypeptide; (2) inserting the nucleic acid molecule into a pET29a expression plasmid comprising a phage T7 promoter and Shine-Dalgarno sequence; (3) transforming E. coli type B strain bacteria BL21/DE3 RIL with the expression plasmid; (4) culturing the transformed bacteria for a time and under conditions sufficient to enable the bacteria to express **pro-UK mutant** polypeptide; and (5) isolating the **pro-UK mutant** polypeptide from the transformed bacteria. The **pro-UK mutant** is non-glycosylated and has a molecular weight of about 45,000 daltons. The culturing comprises a two-stage fermentation. The first stage of fermentation comprises adding to a flask a cell culture diluted in sterile EC1 medium and growing the culture at about 34 to 37 degrees C for at least about 10 hours with agitation to form a seed culture, where the cell culture comprises a glycerol suspension of an LB culture of the transformed bacteria and containing a sufficient amount of kanamycin. The second stage of fermentation comprises: (1) adding the seed culture to a fermenter; (2) maintaining the pH in the fermenter at about 6.8 to 7.2; (3) maintaining the

dissolved oxygen concentration in the culture medium at about 35 to 45% of air saturation; (4) maintaining the temperature of fermentation at about 34 to 37 degrees C; and (5) adding to the fermentor a nutrient feeding solution comprising one or more sugars when all glucose initially present in the fermentor at step (1) is consumed following the equation V = Vo e18t. V = is volume of feeding solution added (ml/h); Vo = is 1/100 of the starting fermentation medium (ml); and t = is time of fermentation after the start of the feeding phase (hours). The expression plasmid containing the nucleic acid molecule is pET29aUKM5. The method further comprises preparing two-chain **pro-UK mutant** by passing the **pro-UK mutant** over plasmin bound to a substrate. The substrate is an agarose-based gel filtration medium. The method further comprises combining the isolated **pro-UK mutant** polypeptide with an acidic excipient. Preparing a pro-urokinase (**pro-UK**) **mutant** polypeptide comprises: (1) obtaining a transformed bacteria, where the bacteria is an E. coli type B strain bacteria BL21/DE3 RIL transformed with a pET29a expression plasmid comprising a phage T7 promoter, a Shine-Dalgarno sequence, and a nucleic acid molecule that encodes a **pro-UK mutant** polypeptide; (2) culturing the transformed bacteria for a time and under conditions sufficient to enable the bacteria to express **pro-UK mutant** polypeptide; and (3) isolating the **pro-UK mutant** polypeptide from the transformed bacteria.

ACTIVITY - Thrombolytic; Cardiant; Cerebroprotective; Vasotropic. No biological data given.

MECHANISM OF ACTION - Gene therapy.

USE - The pro-urokinase (**pro-UK**) **mutant** is useful in clearing a lumen of blood clots or for lysing occlusive thrombi and emboli for treating a person with symptoms of stroke or heart attack in a patient before, during or after surgery. The composition comprising an aliquot of 20 to 40 mg of a **pro-UK mutant**, packaged with directions is useful in administering as a bolus or by infusion to a patient exhibiting symptoms of a stroke or a heart attack. (All claimed.)

ADMINISTRATION - The composition is administered via intravenous route. No biological data given.

EXAMPLE - No relevant examples given. (90 pages)

ACCESSION NUMBER: 2004-26514 BIOTECHDS
TITLE: Use of pro-urokinase (**pro-UK**) **mutant** in clearing a lumen of blood clots for treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery;
recombinant protein production via plasmid expression in host cell for use in disease therapy and gene therapy
AUTHOR: GUREWICH V; WILLIAMS J N; LIU J; SARMIENTOS P; PAGANI M
PATENT ASSIGNEE: THROMBOLYTIC SCI INC
PATENT INFO: WO 2004093797 4 Nov 2004
APPLICATION INFO: WO 2004-US11840 16 Apr 2004
PRIORITY INFO: US 2003-464003 18 Apr 2003; US 2003-463930 18 Apr 2003
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-775860 [76]

=> s (BL21/DE3 RIL)
MISSING OPERATOR

=> s "BL21-DE3-RIL"
L6 20 "BL21-DE3-RIL"

=> s l1 and l6

L7 4 L1 AND L6

=> d l7 ti abs ibib tot

L7 ANSWER 1 OF 4 USPATFULL on STN
TI Methods, devices, and compositions for lysis of occlusive blood clots while sparing wound sealing clots
AB It has now been discovered that certain mutant forms of pro-urokinase ("pro-UK"), such as so-called **pro-UK mutant** "M5" (Lys.sup.300→His), perform in the manner of pro-UK in lysing "bad" blood clots (those clots that occlude blood vessels), while sparing hemostatic fibrin in the so-called "good" blood clots (those clots that seal wounds, e.g., after surgery or other tissue injury). Thus, these pro-UK mutants are excellent and safe thrombolytic agents. These advantages allow them to be used in a variety of new methods, devices, and compositions useful for thrombolysis and treating various cardiovascular disorders in clinical situations where administration of other known thrombolytic agents has been too risky or even contraindicated.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2005:36932 USPATFULL
TITLE: Methods, devices, and compositions for lysis of occlusive blood clots while sparing wound sealing clots
INVENTOR(S): Gurewich, Victor, Cambridge, MA, UNITED STATES
Williams, John N., Boston, MA, UNITED STATES
Liu, Jian-Ning, Brighton, MA, UNITED STATES
Sarmientos, Paolo, Lecco, ITALY
Pagani, Massimiliano, Castelli Calepio (Bergamo), ITALY

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2005031607	A1	20050210	
APPLICATION INFO.:	US 2004-826826	A1	20040416	(10)

	NUMBER	DATE	
PRIORITY INFORMATION:	US 2003-464003P	20030418	(60)
	US 2003-463930P	20030418	(60)
	US 2003-464002P	20030418	(60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: FISH & RICHARDSON PC, 225 FRANKLIN ST, BOSTON, MA, 02110
NUMBER OF CLAIMS: 19
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 19 Drawing Page(s)
LINE COUNT: 2422
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 2 OF 4 USPATFULL on STN
TI Methods of making pro-urokinase mutants
AB Methods are provided for producing non-glycosylated, single-chain and two-chain pro-urokinase (pro-UK) mutants. The methods include cultivating a specific E. coli type B strain that has been transformed with specific plasmids carrying a cDNA sequence that encodes pro-UK mutants and carries specific promoter sequences. Products produced by the methods have medical use for thrombolysis performed while sparing hemostatic clots, e.g., for particular applications such as after a stroke or heart attack.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER: 2005:23319 USPATFULL

TITLE: Methods of making pro-urokinase mutants
INVENTOR(S): Sarmientos, Paolo, Leoco, ITALY
Pagani, Massimiliano, Cividino, ITALY

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2005019863	A1	20050127
APPLICATION INFO.:	US 2004-826598	A1	20040416 (10)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2003-463930P	20030418 (60)
	US 2003-464003P	20030418 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: FISH & RICHARDSON PC, 225 FRANKLIN ST, BOSTON, MA, 02110
NUMBER OF CLAIMS: 24
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 4 Drawing Page(s)
LINE COUNT: 849
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 3 OF 4 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN
TI Use of pro-urokinase (**pro-UK**) **mutant** in clearing a lumen of blood clots for treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery.
AN 2004-775860 [76] WPIDS
AB WO2004093797 A UPAB: 20041125
NOVELTY - A pro-urokinase (**pro-UK**) **mutant** is useful in treating a person with symptoms of stroke or heart attack which comprises determining that the person potentially has had a stroke or heart attack based on observing one or more symptoms of stroke or heart attack and administering to the person a composition comprising an amount of the **pro-UK mutant** effective to lyse any potential blood clot causing the symptoms of stroke or heart attack, is new.

DETAILED DESCRIPTION - A pro-urokinase (**pro-UK**) **mutant** is useful in treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery. INDEPENDENT CLAIMS are also included for:

(1) an intravascular expandable catheter for delivering to a vascular site in a patient an activated, two-chain pro-urokinase (tcpro-UK) mutant;

(2) an intravascular device for delivering to a vascular site in a patient an activated, two-chain pro-urokinase (tcpro-UK) mutant comprising a body and a carrier layer arranged on a surface of the body, where the carrier layer comprises a sustained release agent that slowly releases over time an amount of a tcpro-UK mutant effective to lyse thrombi or emboli in contact with the body;

(3) a method of preparing a pro-urokinase (**pro-UK**) **mutant** polypeptide;

(4) a composition comprising an isolated, single-chain pro-urokinase (**pro-UK mutant** polypeptide, where at least 96% of the protein in the composition is the single-chain **pro-UK mutant** polypeptide;

(5) a purified culture of E. coli type B strain bacteria **BL21 /DE3 RIL**, where bacteria in the culture comprise an expression plasmid encoding a pro-urokinase flexible loop mutant polypeptide; and

(6) preparing a pro-urokinase (**pro-UK**) **mutant** polypeptide.

ACTIVITY - Thrombolytic; Cardiant; Cerebroprotective; Vasotropic.

No biological data given.

MECHANISM OF ACTION - Gene therapy.

USE - The pro-urokinase (**pro-UK**) **mutant** is useful in clearing a lumen of blood clots or for lysing occlusive thrombi and emboli for treating a person with symptoms of stroke or heart attack in a patient before, during or after surgery. The composition comprising an aliquot of 20 to 40 mg of a **pro-UK mutant**, packaged with directions is useful in administering as a bolus or by infusion to a patient exhibiting symptoms of a stroke or a heart attack. (All claimed.)

Dwg.0/14

ACCESSION NUMBER: 2004-775860 [76] WPIDS
DOC. NO. CPI: C2004-271684
TITLE: Use of pro-urokinase (**pro-UK**) **mutant** in clearing a lumen of blood clots for treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery.
DERWENT CLASS: B04 D16 P34
INVENTOR(S): GUREWICH, V; LIU, J; PAGANI, M; SARMIENTOS, P; WILLIAMS, J N
PATENT ASSIGNEE(S): (VLII-N) VLI INT LTD UK; (PAGA-I) PAGANI M; (SARM-I) SARMIENTOS P; (GURE-I) GUREWICH V; (LIUJ-I) LIU J; (WILL-I) WILLIAMS J N; (THRO-N) THROMBOLYTIC SCI INC
COUNTRY COUNT: 108
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2004093797	A2	20041104	(200476)*	EN	90
RW: AT BE BG BW CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE LS LU MC MW MZ NL OA PL PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW					
W: AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NA NI NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL SY TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW					
CA 2426115	A1	20041018	(200476)	EN	
US 2005019863	A1	20050127	(200509)		
US 2005031607	A1	20050210	(200512)		

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
WO 2004093797	A2		WO 2004-US11840	20040416
CA 2426115	A1		CA 2003-2426115	20030422
US 2005019863	A1	Provisional	US 2003-463930P	20030418
		Provisional	US 2003-464003P	20030418
			US 2004-826598	20040416
US 2005031607	A1	Provisional	US 2003-463930P	20030418
		Provisional	US 2003-464002P	20030418
		Provisional	US 2003-464003P	20030418
			US 2004-826826	20040416

PRIORITY APPLN. INFO: US 2003-464003P 20030418; US 2003-463930P 20030418; US 2003-464002P 20030418; US 2004-826598 20040416; US 2004-826826 20040416

L7 ANSWER 4 OF 4 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN

TI Use of pro-urokinase (**pro-UK**) **mutant** in clearing a lumen of blood clots for treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery;
recombinant protein production via plasmid expression in host cell for use in disease therapy and gene therapy

AN 2004-26514 BIOTECHDS

AB DERWENT ABSTRACT:
NOVELTY - A pro-urokinase (**pro-UK**) **mutant** is useful in treating a person with symptoms of stroke or heart attack which comprises determining that the person potentially has had a stroke or heart attack based on observing one or more symptoms of stroke or heart attack and administering to the person a composition comprising an amount of the **pro-UK mutant** effective to lyse any potential blood clot causing the symptoms of stroke or heart attack, is new.

DETAILED DESCRIPTION - A pro-urokinase (**pro-UK**) **mutant** is useful in treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery. INDEPENDENT CLAIMS are also included for: (1) an intravascular expandable catheter for delivering to a vascular site in a patient an activated, two-chain pro-urokinase (tcpro-UK) mutant; (2) an intravascular device for delivering to a vascular site in a patient an activated, two-chain pro-urokinase (tcpro-UK) mutant comprising a body and a carrier layer arranged on a surface of the body, where the carrier layer comprises a sustained release agent that slowly releases over time an amount of a tcpro-UK mutant effective to lyse thrombi or emboli in contact with the body; (3) a method of preparing a pro-urokinase (**pro-UK**) **mutant** polypeptide; (4) a composition comprising an isolated, single-chain pro-urokinase (**pro-UK mutant** polypeptide, where at least 96% of the protein in the composition is the single-chain **pro-UK mutant** polypeptide; (5) a purified culture of E. coli type B strain bacteria **BL21/DE3 RIL**, where bacteria in the culture comprise an expression plasmid encoding a pro-urokinase flexible loop mutant polypeptide; and (6) preparing a pro-urokinase (**pro-UK**) **mutant** polypeptide.

BIOTECHNOLOGY - Preferred Mutant: The new pro-urokinase (**pro-UK**) **mutant** is an activated, two-chain pro-urokinase (tcpro-UK) mutant for clearing a lumen of blood clots, which comprises obtaining a lumen that contains or may contain a blood clot and flowing through the lumen a solution comprising an activated, tcpro-UK mutant for a time sufficient for any blood clots to be dissolved. The solution comprises a concentration of tcpro-UK mutant of 0.05-0.2 mg. The lumen is in a catheter, blood pump or artificial organ. The tcproUK mutant is a low molecular weight tcpro-UK mutant. The new pro-urokinase (**pro-UK**) **mutant** is useful in treating a person with symptoms of stroke or heart attack which comprises determining that the person potentially has had a stroke or heart attack based on observing one or more symptoms of stroke or heart attack and administering to the person a composition comprising an amount of the **pro-UK mutant** effective to lyse any potential blood clot causing the symptoms of stroke or heart attack. The pro-urokinase (**pro-UK**) **mutant** is useful in lysing occlusive thrombi and emboli in a patient before, during, or after surgery, which comprises administering to the patient within 5 hours before surgery, during surgery, or within 24 hours after surgery, a composition comprising the **pro-UK mutant** effective to preferentially lyse any potential occlusive thrombus or embolus compared to hemostatic fibrin in wound sealing clots. The **pro-UK mutant** comprises a pro-UK flexible loop mutant. The **pro-UK mutant** comprises the mutation Lys300 to His. The

composition is administered more than 3 hours after the onset of symptoms. A bolus of the composition comprising 20-50 mg of the **pro-UK mutant** is administered. The method further comprises obtaining a medical confirmation of an occlusive thrombus in the brain and administering an infusion of the composition at a **pro-UK mutant** dosage of dose of 120-200 mg/hour (intravenous) or 50-100 mg/hour (intra-arterial). The composition is administered within 90 minutes of the onset of symptoms. The pro-urokinase (**pro-UK**) **mutant** is useful in lysing occlusive thrombi and emboli in a patient before, during, or after surgery, which comprises administering to the patient within 5 hours before surgery, during surgery, or within 24 hours after surgery, a composition comprising the **pro-UK mutant** effective to preferentially lyse any potential occlusive thrombus or embolus compared to hemostatic fibrin in wound sealing clots. The composition is administered by infusion within three hours before, during or after surgery. The composition is administered by infusion at a **pro-UK mutant** dosage of 50 - 200 ml/hour.

Preferred Catheter: The intravascular expandable catheter for delivering to a vascular site in a patient an activated, two-chain pro-urokinase (tcpro-UK) mutant comprises: (1) a catheter body having proximal and distal ends; (2) an expandable portion arranged at the distal end of the catheter body; and (3) a carrier layer arranged on a surface of the expandable portion, where the carrier layer comprises an amount of a tcpro-UK mutant effective to lyse thrombi or emboli in contact with the expandable portion. The carrier layer is a hydrogel selected to quickly release effective amounts of the tcpro-UK mutant upon contact with a thrombus or embolus. The amount of the tcpro-UK mutant comprises 0.1-0.5 mg. The carrier layer comprises a lumen containing the tcpro-UK mutant and one or more apertures that are pressed against a thrombus or embolus to allow the thrombus or embolus to protrude into the one or more apertures, thereby contacting the tcpro-UK mutant. The carrier layer comprises activated, two-chain **pro-UK mutant**

M5. Preferred Device: The device is a stent or suture. Preferred Composition: The composition comprises an isolated, single-chain pro-urokinase (**pro-UK**) **mutant** polypeptide, where at least 98% of the protein in the composition is the single-chain **pro-UK mutant** polypeptide, and an acidic excipient. Preferred Method: Preparing a pro-urokinase (**pro-UK**) **mutant** polypeptide comprises: (1) obtaining a nucleic acid molecule that encodes a **pro-UK mutant** polypeptide; (2) inserting the nucleic acid molecule into a pET29a expression plasmid comprising a phage T7 promoter and Shine-Dalgarno sequence; (3) transforming E. coli type B strain bacteria **BL21/DE3 RIL** with the expression plasmid; (4) culturing the transformed bacteria for a time and under conditions sufficient to enable the bacteria to express **pro-UK mutant** polypeptide; and (5) isolating the **pro-UK mutant** polypeptide from the transformed bacteria.

The **pro-UK mutant** is non-glycosylated and has a molecular weight of about 45,000 daltons. The culturing comprises a two-stage fermentation. The first stage of fermentation comprises adding to a flask a cell culture diluted in sterile EC1 medium and growing the culture at about 34 to 37 degrees C for at least about 10 hours with agitation to form a seed culture, where the cell culture comprises a glycerol suspension of an LB culture of the transformed bacteria and containing a sufficient amount of kanamycin. The second stage of fermentation comprises: (1) adding the seed culture to a fermenter; (2) maintaining the pH in the fermenter at about 6.8 to 7.2; (3) maintaining the dissolved oxygen concentration in the culture medium at about 35 to 45% of air saturation; (4) maintaining the temperature of fermentation at about 34 to 37 degrees C; and (5) adding to the fermentor a nutrient feeding solution comprising one or more sugars when all glucose initially

present in the fermentor at step (1) is consumed following the equation V = Vo e18t. V = is volume of feeding solution added (ml/h); Vo = is 1/100 of the starting fermentation medium (ml); and t = is time of fermentation after the start of the feeding phase (hours). The expression plasmid containing the nucleic acid molecule is pET29aUKM5. The method further comprises preparing two-chain **pro-UK mutant** by passing the **pro-UK mutant** over plasmin bound to a substrate. The substrate is an agarose-based gel filtration medium. The method further comprises combining the isolated **pro-UK mutant** polypeptide with an acidic excipient. Preparing a pro-urokinase (**pro-UK**) **mutant** polypeptide comprises: (1) obtaining a transformed bacteria, where the bacteria is an E. coli type B strain bacteria **BL21/DE3 RIL** transformed with a pET29a expression plasmid comprising a phage T7 promoter, a Shine-Dalgarno sequence, and a nucleic acid molecule that encodes a **pro-UK mutant** polypeptide; (2) culturing the transformed bacteria for a time and under conditions sufficient to enable the bacteria to express **pro-UK mutant** polypeptide; and (3) isolating the **pro-UK mutant** polypeptide from the transformed bacteria.

ACTIVITY - Thrombolytic; Cardiant; Cerebroprotective; Vasotropic. No biological data given.

MECHANISM OF ACTION - Gene therapy.

USE - The pro-urokinase (**pro-UK**) **mutant** is useful in clearing a lumen of blood clots or for lysing occlusive thrombi and emboli for treating a person with symptoms of stroke or heart attack in a patient before, during or after surgery. The composition comprising an aliquot of 20 to 40 mg of a **pro-UK mutant**, packaged with directions is useful in administering as a bolus or by infusion to a patient exhibiting symptoms of a stroke or a heart attack. (All claimed.)

ADMINISTRATION - The composition is administered via intravenous route. No biological data given.

EXAMPLE - No relevant examples given. (90 pages)

ACCESSION NUMBER: 2004-26514 BIOTECHDS
TITLE: Use of pro-urokinase (**pro-UK**) **mutant** in clearing a lumen of blood clots for treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery;
recombinant protein production via plasmid expression in host cell for use in disease therapy and gene therapy
AUTHOR: GUREWICH V; WILLIAMS J N; LIU J; SARMIENTOS P; PAGANI M
PATENT ASSIGNEE: THROMBOLYTIC SCI INC
PATENT INFO: WO 2004093797 4 Nov 2004
APPLICATION INFO: WO 2004-US11840 16 Apr 2004
PRIORITY INFO: US 2003-464003 18 Apr 2003; US 2003-463930 18 Apr 2003
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-775860 [76]

=> d his

(FILE 'HOME' ENTERED AT 14:24:41 ON 18 NOV 2005)

FILE 'MEDLINE, USPATFULL, DGENE, EMBASE, WPIDS, FSTA, JICST-EPLUS, BIOSIS, BIOTECHDS' ENTERED AT 14:25:14 ON 18 NOV 2005
L1 43 S (PRO-UROKINASE MUTANT OR PRO-UK MUTANT)
L2 0 S L1 AND (PREPARATION METHOD)
L3 8 S L1 AND M5
L4 6 S (E COLI TYPE B STRAIN)

```
L5             2 S L1 AND (LYS300 TO HIS)
L6            20 S "BL21-DE3-RIL"
L7             4 S L1 AND L6

=> s l6 and (M5)
L8             4 L6 AND (M5)

=> d l8 ti abs ibib tot

L8   ANSWER 1 OF 4  USPATFULL on STN
TI     Methods, devices, and compositions for lysis of occlusive blood clots
       while sparing wound sealing clots
AB     It has now been discovered that certain mutant forms of pro-urokinase
       ("pro-UK"), such as so-called pro-UK mutant "M5"
       (Lys.sup.300→His), perform in the manner of pro-UK in lysing
       "bad" blood clots (those clots that occlude blood vessels), while
       sparing hemostatic fibrin in the so-called "good" blood clots (those
       clots that seal wounds, e.g., after surgery or other tissue injury).
       Thus, these pro-UK mutants are excellent and safe thrombolytic agents.
       These advantages allow them to be used in a variety of new methods,
       devices, and compositions useful for thrombolysis and treating various
       cardiovascular disorders in clinical situations where administration of
       other known thrombolytic agents has been too risky or even
       contraindicated.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.
ACCESSION NUMBER:        2005:36932  USPATFULL
TITLE:                   Methods, devices, and compositions for lysis of
                         occlusive blood clots while sparing wound sealing clots
INVENTOR(S):             Gurewich, Victor, Cambridge, MA, UNITED STATES
                         Williams, John N., Boston, MA, UNITED STATES
                         Liu, Jian-Ning, Brighton, MA, UNITED STATES
                         Sarmientos, Paolo, Lecco, ITALY
                         Pagani, Massimiliano, Castelli Calepio (Bergamo), ITALY

                              NUMBER         KIND    DATE
                         --------------- ------ --------
PATENT INFORMATION:      US 2005031607      A1   20050210
APPLICATION INFO.:       US 2004-826826     A1   20040416  (10)

                                NUMBER         DATE
                         ------------------ --------
PRIORITY INFORMATION:    US 2003-464003P    20030418 (60)
                         US 2003-463930P    20030418 (60)
                         US 2003-464002P    20030418 (60)
DOCUMENT TYPE:           Utility
FILE SEGMENT:            APPLICATION
LEGAL REPRESENTATIVE:    FISH & RICHARDSON PC, 225 FRANKLIN ST, BOSTON, MA,
                         02110
NUMBER OF CLAIMS:        19
EXEMPLARY CLAIM:         1
NUMBER OF DRAWINGS:      19 Drawing Page(s)
LINE COUNT:              2422
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8   ANSWER 2 OF 4  USPATFULL on STN
TI     Methods of making pro-urokinase mutants
AB     Methods are provided for producing non-glycosylated, single-chain and
       two-chain pro-urokinase (pro-UK) mutants. The methods include
       cultivating a specific E. coli type B strain that has been transformed
       with specific plasmids carrying a cDNA sequence that encodes pro-UK
       mutants and carries specific promoter sequences. Products produced by
       the methods have medical use for thrombolysis performed while sparing
```

hemostatic clots, e.g., for particular applications such as after a stroke or heart attack.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2005:23319 USPATFULL		
TITLE:	Methods of making pro-urokinase mutants		
INVENTOR(S):	Sarmientos, Paolo, Leoco, ITALY Pagani, Massimiliano, Cividino, ITALY		

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2005019863	A1	20050127
APPLICATION INFO.:	US 2004-826598	A1	20040416 (10)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2003-463930P	20030418 (60)
	US 2003-464003P	20030418 (60)

DOCUMENT TYPE:	Utility
FILE SEGMENT:	APPLICATION
LEGAL REPRESENTATIVE:	FISH & RICHARDSON PC, 225 FRANKLIN ST, BOSTON, MA, 02110
NUMBER OF CLAIMS:	24
EXEMPLARY CLAIM:	1
NUMBER OF DRAWINGS:	4 Drawing Page(s)
LINE COUNT:	849

CAS.INDEXING IS AVAILABLE FOR THIS PATENT.

L8 ANSWER 3 OF 4 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN

TI Use of pro-urokinase (pro-UK) mutant in clearing a lumen of blood clots for treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery.

AN 2004-775860 [76] WPIDS

AB WO2004093797 A UPAB: 20041125

NOVELTY - A pro-urokinase (pro-UK) mutant is useful in treating a person with symptoms of stroke or heart attack which comprises determining that the person potentially has had a stroke or heart attack based on observing one or more symptoms of stroke or heart attack and administering to the person a composition comprising an amount of the pro-UK mutant effective to lyse any potential blood clot causing the symptoms of stroke or heart attack, is new.

DETAILED DESCRIPTION - A pro-urokinase (pro-UK) mutant is useful in treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery.

INDEPENDENT CLAIMS are also included for:

(1) an intravascular expandable catheter for delivering to a vascular site in a patient an activated, two-chain pro-urokinase (tcpro-UK) mutant;

(2) an intravascular device for delivering to a vascular site in a patient an activated, two-chain pro-urokinase (tcpro-UK) mutant comprising a body and a carrier layer arranged on a surface of the body, where the carrier layer comprises a sustained release agent that slowly releases over time an amount of a tcpro-UK mutant effective to lyse thrombi or emboli in contact with the body;

(3) a method of preparing a pro-urokinase (pro-UK) mutant polypeptide;

(4) a composition comprising an isolated, single-chain pro-urokinase (pro-UK mutant polypeptide, where at least 96% of the protein in the composition is the single-chain pro-UK mutant polypeptide;

(5) a purified culture of E. coli type B strain bacteria **BL21 /DE3 RIL**, where bacteria in the culture comprise an expression plasmid encoding a pro-urokinase flexible loop mutant polypeptide; and

(6) preparing a pro-urokinase (pro-UK) mutant polypeptide.

ACTIVITY - Thrombolytic; Cardiant; Cerebroprotective; Vasotropic.
No biological data given.
MECHANISM OF ACTION - Gene therapy.
USE - The pro-urokinase (pro-UK) mutant is useful in clearing a lumen of blood clots or for lysing occlusive thrombi and emboli for treating a person with symptoms of stroke or heart attack in a patient before, during or after surgery. The composition comprising an aliquot of 20 to 40 mg of a pro-UK mutant, packaged with directions is useful in administering as a bolus or by infusion to a patient exhibiting symptoms of a stroke or a heart attack. (All claimed.)
Dwg.0/14

ACCESSION NUMBER: 2004-775860 [76] WPIDS
DOC. NO. CPI: C2004-271684
TITLE: Use of pro-urokinase (pro-UK) mutant in clearing a lumen of blood clots for treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery.
DERWENT CLASS: B04 D16 P34
INVENTOR(S): GUREWICH, V; LIU, J; PAGANI, M; SARMIENTOS, P; WILLIAMS, J N
PATENT ASSIGNEE(S): (VLII-N) VLI INT LTD UK; (PAGA-I) PAGANI M; (SARM-I) SARMIENTOS P; (GURE-I) GUREWICH V; (LIUJ-I) LIU J; (WILL-I) WILLIAMS J N; (THRO-N) THROMBOLYTIC SCI INC
COUNTRY COUNT: 108
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2004093797	A2	20041104	(200476)*	EN	90
RW: AT BE BG BW CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE LS LU MC MW MZ NL OA PL PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW					
W: AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NA NI NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL SY TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW					
CA 2426115	A1	20041018	(200476)	EN	
US 2005019863	A1	20050127	(200509)		
US 2005031607	A1	20050210	(200512)		

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
WO 2004093797	A2		WO 2004-US11840	20040416
CA 2426115	A1		CA 2003-2426115	20030422
US 2005019863	A1	Provisional	US 2003-463930P	20030418
		Provisional	US 2003-464003P	20030418
			US 2004-826598	20040416
US 2005031607	A1	Provisional	US 2003-463930P	20030418
		Provisional	US 2003-464002P	20030418
		Provisional	US 2003-464003P	20030418
			US 2004-826826	20040416

PRIORITY APPLN. INFO: US 2003-464003P 20030418; US 2003-463930P 20030418; US 2003-464002P 20030418; US 2004-826598 20040416; US 2004-826826 20040416

L8 ANSWER 4 OF 4 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
TI Use of pro-urokinase (pro-UK) mutant in clearing a lumen of blood clots

for treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery;

recombinant protein production via plasmid expression in host cell for use in disease therapy and gene therapy

AN 2004-26514 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - A pro-urokinase (pro-UK) mutant is useful in treating a person with symptoms of stroke or heart attack which comprises determining that the person potentially has had a stroke or heart attack based on observing one or more symptoms of stroke or heart attack and administering to the person a composition comprising an amount of the pro-UK mutant effective to lyse any potential blood clot causing the symptoms of stroke or heart attack, is new.

DETAILED DESCRIPTION - A pro-urokinase (pro-UK) mutant is useful in treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery. INDEPENDENT CLAIMS are also included for: (1) an intravascular expandable catheter for delivering to a vascular site in a patient an activated, two-chain pro-urokinase (tcpro-UK) mutant; (2) an intravascular device for delivering to a vascular site in a patient an activated, two-chain pro-urokinase (tcpro-UK) mutant comprising a body and a carrier layer arranged on a surface of the body, where the carrier layer comprises a sustained release agent that slowly releases over time an amount of a tcpro-UK mutant effective to lyse thrombi or emboli in contact with the body; (3) a method of preparing a pro-urokinase (pro-UK) mutant polypeptide; (4) a composition comprising an isolated, single-chain pro-urokinase (pro-UK mutant polypeptide, where at least 96% of the protein in the composition is the single-chain pro-UK mutant polypeptide; (5) a purified culture of E. coli type B strain bacteria **BL21/DE3 RIL**, where bacteria in the culture comprise an expression plasmid encoding a pro-urokinase flexible loop mutant polypeptide; and (6) preparing a pro-urokinase (pro-UK) mutant polypeptide.

BIOTECHNOLOGY - Preferred Mutant: The new pro-urokinase (pro-UK) mutant is an activated, two-chain pro-urokinase (tcpro-UK) mutant for clearing a lumen of blood clots, which comprises obtaining a lumen that contains or may contain a blood clot and flowing through the lumen a solution comprising an activated, tcpro-UK mutant for a time sufficient for any blood clots to be dissolved. The solution comprises a concentration of tcpro-UK mutant of 0.05-0.2 mg. The lumen is in a catheter, blood pump or artificial organ. The tcproUK mutant is a low molecular weight tcpro-UK mutant. The new pro-urokinase (pro-UK) mutant is useful in treating a person with symptoms of stroke or heart attack which comprises determining that the person potentially has had a stroke or heart attack based on observing one or more symptoms of stroke or heart attack and administering to the person a composition comprising an amount of the pro-UK mutant effective to lyse any potential blood clot causing the symptoms of stroke or heart attack. The pro-urokinase (pro-UK) mutant is useful in lysing occlusive thrombi and emboli in a patient before, during, or after surgery, which comprises administering to the patient within 5 hours before surgery, during surgery, or within 24 hours after surgery, a composition comprising the pro-UK mutant effective to preferentially lyse any potential occlusive thrombus or embolus compared to hemostatic fibrin in wound sealing clots. The pro-UK mutant comprises a pro-UK flexible loop mutant. The pro-UK mutant comprises the mutation Lys300 to His. The composition is administered more than 3 hours after the onset of symptoms. A bolus of the composition comprising 20-50 mg of the pro-UK mutant is administered. The method further comprises obtaining a medical confirmation of an occlusive thrombus in the brain and administering an infusion of the composition at a pro-UK mutant dosage of dose of 120-200 mg/hour (intravenous) or 50-100 mg/hour (intra-arterial). The composition is administered within 90

minutes of the onset of symptoms. The pro-urokinase (pro-UK) mutant is useful in lysing occlusive thrombi and emboli in a patient before, during, or after surgery, which comprises administering to the patient within 5 hours before surgery, during surgery, or within 24 hours after surgery, a composition comprising the pro-UK mutant effective to preferentially lyse any potential occlusive thrombus or embolus compared to hemostatic fibrin in wound sealing clots. The composition is administered by infusion within three hours before, during or after surgery. The composition is administered by infusion at a pro-UK mutant dosage of 50 - 200 ml/hour. Preferred Catheter: The intravascular expandable catheter for delivering to a vascular site in a patient an activated, two-chain pro-urokinase (tcpro-UK) mutant comprises: (1) a catheter body having proximal and distal ends; (2) an expandable portion arranged at the distal end of the catheter body; and (3) a carrier layer arranged on a surface of the expandable portion, where the carrier layer comprises an amount of a tcpro-UK mutant effective to lyse thrombi or emboli in contact with the expandable portion. The carrier layer is a hydrogel selected to quickly release effective amounts of the tcpro-UK mutant upon contact with a thrombus or embolus. The amount of the tcpro-UK mutant comprises 0.1-0.5 mg. The carrier layer comprises a lumen containing the tcpro-UK mutant and one or more apertures that are pressed against a thrombus or embolus to allow the thrombus or embolus to protrude into the one or more apertures, thereby contacting the tcpro-UK mutant. The carrier layer comprises activated, two-chain pro-UK mutant **M5**. Preferred Device: The device is a stent or suture. Preferred Composition: The composition comprises an isolated, single-chain pro-urokinase (pro-UK) mutant polypeptide, where at least 98% of the protein in the composition is the single-chain pro-UK mutant polypeptide, and an acidic excipient. Preferred Method: Preparing a pro-urokinase (pro-UK) mutant polypeptide comprises: (1) obtaining a nucleic acid molecule that encodes a pro-UK mutant polypeptide; (2) inserting the nucleic acid molecule into a pET29a expression plasmid comprising a phage T7 promoter and Shine-Dalgarno sequence; (3) transforming E. coli type B strain bacteria **BL21/DE3 RIL** with the expression plasmid; (4) culturing the transformed bacteria for a time and under conditions sufficient to enable the bacteria to express pro-UK mutant polypeptide; and (5) isolating the pro-UK mutant polypeptide from the transformed bacteria. The pro-UK mutant is non-glycosylated and has a molecular weight of about 45,000 daltons. The culturing comprises a two-stage fermentation. The first stage of fermentation comprises adding to a flask a cell culture diluted in sterile EC1 medium and growing the culture at about 34 to 37 degrees C for at least about 10 hours with agitation to form a seed culture, where the cell culture comprises a glycerol suspension of an LB culture of the transformed bacteria and containing a sufficient amount of kanamycin. The second stage of fermentation comprises: (1) adding the seed culture to a fermenter; (2) maintaining the pH in the fermenter at about 6.8 to 7.2; (3) maintaining the dissolved oxygen concentration in the culture medium at about 35 to 45% of air saturation; (4) maintaining the temperature of fermentation at about 34 to 37 degrees C; and (5) adding to the fermentor a nutrient feeding solution comprising one or more sugars when all glucose initially present in the fermentor at step (1) is consumed following the equation V = Vo e18t. V = is volume of feeding solution added (ml/h); Vo = is 1/100 of the starting fermentation medium (ml); and t = is time of fermentation after the start of the feeding phase (hours). The expression plasmid containing the nucleic acid molecule is pET29aUKM5. The method further comprises preparing two-chain pro-UK mutant by passing the pro-UK mutant over plasmin bound to a substrate. The substrate is an agarose-based gel filtration medium. The method further comprises combining the isolated pro-UK mutant polypeptide with an acidic excipient. Preparing a pro-urokinase (pro-UK) mutant polypeptide comprises: (1) obtaining a transformed bacteria, where the bacteria is an E. coli type B strain bacteria **BL21/DE3 RIL**

transformed with a pET29a expression plasmid comprising a phage T7 promoter, a Shine-Dalgarno sequence, and a nucleic acid molecule that encodes a pro-UK mutant polypeptide; (2) culturing the transformed bacteria for a time and under conditions sufficient to enable the bacteria to express pro-UK mutant polypeptide; and (3) isolating the pro-UK mutant polypeptide from the transformed bacteria.

ACTIVITY - Thrombolytic; Cardiant; Cerebroprotective; Vasotropic. No biological data given.

MECHANISM OF ACTION - Gene therapy.

USE - The pro-urokinase (pro-UK) mutant is useful in clearing a lumen of blood clots or for lysing occlusive thrombi and emboli for treating a person with symptoms of stroke or heart attack in a patient before, during or after surgery. The composition comprising an aliquot of 20 to 40 mg of a pro-UK mutant, packaged with directions is useful in administering as a bolus or by infusion to a patient exhibiting symptoms of a stroke or a heart attack. (All claimed.)

ADMINISTRATION - The composition is administered via intravenous route. No biological data given.

EXAMPLE - No relevant examples given. (90 pages)

ACCESSION NUMBER: 2004-26514 BIOTECHDS
TITLE: Use of pro-urokinase (pro-UK) mutant in clearing a lumen of blood clots for treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery;
recombinant protein production via plasmid expression in host cell for use in disease therapy and gene therapy
AUTHOR: GUREWICH V; WILLIAMS J N; LIU J; SARMIENTOS P; PAGANI M
PATENT ASSIGNEE: THROMBOLYTIC SCI INC
PATENT INFO: WO 2004093797 4 Nov 2004
APPLICATION INFO: WO 2004-US11840 16 Apr 2004
PRIORITY INFO: US 2003-464003 18 Apr 2003; US 2003-463930 18 Apr 2003
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-775860 [76]

=> d his

(FILE 'HOME' ENTERED AT 14:24:41 ON 18 NOV 2005)

FILE 'MEDLINE, USPATFULL, DGENE, EMBASE, WPIDS, FSTA, JICST-EPLUS, BIOSIS, BIOTECHDS' ENTERED AT 14:25:14 ON 18 NOV 2005

L1 43 S (PRO-UROKINASE MUTANT OR PRO-UK MUTANT)
L2 0 S L1 AND (PREPARATION METHOD)
L3 8 S L1 AND M5
L4 6 S (E COLI TYPE B STRAIN)
L5 2 S L1 AND (LYS300 TO HIS)
L6 20 S "BL21-DE3-RIL"
L7 4 S L1 AND L6
L8 4 S L6 AND (M5)

=> s l6 and (T7 promoter)
L9 4 L6 AND (T7 PROMOTER)

=> d l9 ti abs ibib tot

L9 ANSWER 1 OF 4 USPATFULL on STN
TI Methods, devices, and compositions for lysis of occlusive blood clots while sparing wound sealing clots
AB It has now been discovered that certain mutant forms of pro-urokinase ("pro-UK"), such as so-called pro-UK mutant "M5" (Lys.sup.300→His), perform in the manner of pro-UK in lysing "bad" blood clots (those clots that occlude blood vessels), while

sparing hemostatic fibrin in the so-called "good" blood clots (those clots that seal wounds, e.g., after surgery or other tissue injury). Thus, these pro-UK mutants are excellent and safe thrombolytic agents. These advantages allow them to be used in a variety of new methods, devices, and compositions useful for thrombolysis and treating various cardiovascular disorders in clinical situations where administration of other known thrombolytic agents has been too risky or even contraindicated.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2005:36932 USPATFULL			
TITLE:	Methods, devices, and compositions for lysis of occlusive blood clots while sparing wound sealing clots			
INVENTOR(S):	Gurewich, Victor, Cambridge, MA, UNITED STATES Williams, John N., Boston, MA, UNITED STATES Liu, Jian-Ning, Brighton, MA, UNITED STATES Sarmientos, Paolo, Lecco, ITALY Pagani, Massimiliano, Castelli Calepio (Bergamo), ITALY			
	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2005031607	A1	20050210	
APPLICATION INFO.:	US 2004-826826	A1	20040416	(10)
	NUMBER		DATE	
PRIORITY INFORMATION:	US 2003-464003P		20030418	(60)
	US 2003-463930P		20030418	(60)
	US 2003-464002P		20030418	(60)
DOCUMENT TYPE:	Utility			
FILE SEGMENT:	APPLICATION			
LEGAL REPRESENTATIVE:	FISH & RICHARDSON PC, 225 FRANKLIN ST, BOSTON, MA, 02110			
NUMBER OF CLAIMS:	19			
EXEMPLARY CLAIM:	1			
NUMBER OF DRAWINGS:	19 Drawing Page(s)			
LINE COUNT:	2422			

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9 ANSWER 2 OF 4 USPATFULL on STN

TI Methods of making pro-urokinase mutants

AB Methods are provided for producing non-glycosylated, single-chain and two-chain pro-urokinase (pro-UK) mutants. The methods include cultivating a specific E. coli type B strain that has been transformed with specific plasmids carrying a cDNA sequence that encodes pro-UK mutants and carries specific promoter sequences. Products produced by the methods have medical use for thrombolysis performed while sparing hemostatic clots, e.g., for particular applications such as after a stroke or heart attack.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

ACCESSION NUMBER:	2005:23319 USPATFULL			
TITLE:	Methods of making pro-urokinase mutants			
INVENTOR(S):	Sarmientos, Paolo, Leoco, ITALY Pagani, Massimiliano, Cividino, ITALY			
	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2005019863	A1	20050127	
APPLICATION INFO.:	US 2004-826598	A1	20040416	(10)
	NUMBER		DATE	

PRIORITY INFORMATION: US 2003-463930P 20030418 (60)
US 2003-464003P 20030418 (60)
DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: FISH & RICHARDSON PC, 225 FRANKLIN ST, BOSTON, MA, 02110
NUMBER OF CLAIMS: 24
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 4 Drawing Page(s)
LINE COUNT: 849
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9 ANSWER 3 OF 4 WPIDS COPYRIGHT 2005 THE THOMSON CORP on STN
TI Use of pro-urokinase (pro-UK) mutant in clearing a lumen of blood clots for treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery.
AN 2004-775860 [76] WPIDS
AB WO2004093797 A UPAB: 20041125
NOVELTY - A pro-urokinase (pro-UK) mutant is useful in treating a person with symptoms of stroke or heart attack which comprises determining that the person potentially has had a stroke or heart attack based on observing one or more symptoms of stroke or heart attack and administering to the person a composition comprising an amount of the pro-UK mutant effective to lyse any potential blood clot causing the symptoms of stroke or heart attack, is new.

DETAILED DESCRIPTION - A pro-urokinase (pro-UK) mutant is useful in treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery.

INDEPENDENT CLAIMS are also included for:

(1) an intravascular expandable catheter for delivering to a vascular site in a patient an activated, two-chain pro-urokinase (tcpro-UK) mutant;

(2) an intravascular device for delivering to a vascular site in a patient an activated, two-chain pro-urokinase (tcpro-UK) mutant comprising a body and a carrier layer arranged on a surface of the body, where the carrier layer comprises a sustained release agent that slowly releases over time an amount of a tcpro-UK mutant effective to lyse thrombi or emboli in contact with the body;

(3) a method of preparing a pro-urokinase (pro-UK) mutant polypeptide;

(4) a composition comprising an isolated, single-chain pro-urokinase (pro-UK mutant polypeptide, where at least 96% of the protein in the composition is the single-chain pro-UK mutant polypeptide;

(5) a purified culture of E. coli type B strain bacteria **BL21 /DE3 RIL**, where bacteria in the culture comprise an expression plasmid encoding a pro-urokinase flexible loop mutant polypeptide; and

(6) preparing a pro-urokinase (pro-UK) mutant polypeptide.

ACTIVITY - Thrombolytic; Cardiant; Cerebroprotective; Vasotropic.
No biological data given.

MECHANISM OF ACTION - Gene therapy.

USE - The pro-urokinase (pro-UK) mutant is useful in clearing a lumen of blood clots or for lysing occlusive thrombi and emboli for treating a person with symptoms of stroke or heart attack in a patient before, during or after surgery. The composition comprising an aliquot of 20 to 40 mg of a pro-UK mutant, packaged with directions is useful in administering as a bolus or by infusion to a patient exhibiting symptoms of a stroke or a heart attack. (All claimed.)

Dwg.0/14
ACCESSION NUMBER: 2004-775860 [76] WPIDS
DOC. NO. CPI: C2004-271684
TITLE: Use of pro-urokinase (pro-UK) mutant in clearing a lumen of blood clots for treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and

emboli in a patient before, during or after surgery.
DERWENT CLASS: B04 D16 P34
INVENTOR(S): GUREWICH, V; LIU, J; PAGANI, M; SARMIENTOS, P; WILLIAMS, J N
PATENT ASSIGNEE(S): (VLII-N) VLI INT LTD UK; (PAGA-I) PAGANI M; (SARM-I) SARMIENTOS P; (GURE-I) GUREWICH V; (LIUJ-I) LIU J; (WILL-I) WILLIAMS J N; (THRO-N) THROMBOLYTIC SCI INC
COUNTRY COUNT: 108
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2004093797	A2	20041104	(200476)*	EN	90
RW: AT BE BG BW CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE LS LU MC MW MZ NL OA PL PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW					
W: AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NA NI NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL SY TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW					
CA 2426115	A1	20041018	(200476)	EN	
US 2005019863	A1	20050127	(200509)		
US 2005031607	A1	20050210	(200512)		

APPLICATION DETAILS:

PATENT NO	KIND		APPLICATION	DATE
WO 2004093797	A2		WO 2004-US11840	20040416
CA 2426115	A1		CA 2003-2426115	20030422
US 2005019863	A1	Provisional	US 2003-463930P	20030418
		Provisional	US 2003-464003P	20030418
			US 2004-826598	20040416
US 2005031607	A1	Provisional	US 2003-463930P	20030418
		Provisional	US 2003-464002P	20030418
		Provisional	US 2003-464003P	20030418
			US 2004-826826	20040416

PRIORITY APPLN. INFO: US 2003-464003P 20030418; US 2003-463930P 20030418; US 2003-464002P 20030418; US 2004-826598 20040416; US 2004-826826 20040416

L9 ANSWER 4 OF 4 BIOTECHDS COPYRIGHT 2005 THE THOMSON CORP. on STN
TI Use of pro-urokinase (pro-UK) mutant in clearing a lumen of blood clots for treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery;
recombinant protein production via plasmid expression in host cell for use in disease therapy and gene therapy
AN 2004-26514 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - A pro-urokinase (pro-UK) mutant is useful in treating a person with symptoms of stroke or heart attack which comprises determining that the person potentially has had a stroke or heart attack based on observing one or more symptoms of stroke or heart attack and administering to the person a composition comprising an amount of the pro-UK mutant effective to lyse any potential blood clot causing the symptoms of stroke or heart attack, is new.
DETAILED DESCRIPTION - A pro-urokinase (pro-UK) mutant is useful in treating a person with symptoms of stroke or heart attack or in lysing

occlusive thrombi and emboli in a patient before, during or after surgery. INDEPENDENT CLAIMS are also included for: (1) an intravascular expandable catheter for delivering to a vascular site in a patient an activated, two-chain pro-urokinase (tcpro-UK) mutant; (2) an intravascular device for delivering to a vascular site in a patient an activated, two-chain pro-urokinase (tcpro-UK) mutant comprising a body and a carrier layer arranged on a surface of the body, where the carrier layer comprises a sustained release agent that slowly releases over time an amount of a tcpro-UK mutant effective to lyse thrombi or emboli in contact with the body; (3) a method of preparing a pro-urokinase (pro-UK) mutant polypeptide; (4) a composition comprising an isolated, single-chain pro-urokinase (pro-UK mutant polypeptide, where at least 96% of the protein in the composition is the single-chain pro-UK mutant polypeptide; (5) a purified culture of E. coli type B strain bacteria **BL21/DE3 RIL**, where bacteria in the culture comprise an expression plasmid encoding a pro-urokinase flexible loop mutant polypeptide; and (6) preparing a pro-urokinase (pro-UK) mutant polypeptide.

BIOTECHNOLOGY - Preferred Mutant: The new pro-urokinase (pro-UK) mutant is an activated, two-chain pro-urokinase (tcpro-UK) mutant for clearing a lumen of blood clots, which comprises obtaining a lumen that contains or may contain a blood clot and flowing through the lumen a solution comprising an activated, tcpro-UK mutant for a time sufficient for any blood clots to be dissolved. The solution comprises a concentration of tcpro-UK mutant of 0.05-0.2 mg. The lumen is in a catheter, blood pump or artificial organ. The tcproUK mutant is a low molecular weight tcpro-UK mutant. The new pro-urokinase (pro-UK) mutant is useful in treating a person with symptoms of stroke or heart attack which comprises determining that the person potentially has had a stroke or heart attack based on observing one or more symptoms of stroke or heart attack and administering to the person a composition comprising an amount of the pro-UK mutant effective to lyse any potential blood clot causing the symptoms of stroke or heart attack. The pro-urokinase (pro-UK) mutant is useful in lysing occlusive thrombi and emboli in a patient before, during, or after surgery, which comprises administering to the patient within 5 hours before surgery, during surgery, or within 24 hours after surgery, a composition comprising the pro-UK mutant effective to preferentially lyse any potential occlusive thrombus or embolus compared to hemostatic fibrin in wound sealing clots. The pro-UK mutant comprises a pro-UK flexible loop mutant. The pro-UK mutant comprises the mutation Lys300 to His. The composition is administered more than 3 hours after the onset of symptoms. A bolus of the composition comprising 20-50 mg of the pro-UK mutant is administered. The method further comprises obtaining a medical confirmation of an occlusive thrombus in the brain and administering an infusion of the composition at a pro-UK mutant dosage of dose of 120-200 mg/hour (intravenous) or 50-100 mg/hour (intra-arterial). The composition is administered within 90 minutes of the onset of symptoms. The pro-urokinase (pro-UK) mutant is useful in lysing occlusive thrombi and emboli in a patient before, during, or after surgery, which comprises administering to the patient within 5 hours before surgery, during surgery, or within 24 hours after surgery, a composition comprising the pro-UK mutant effective to preferentially lyse any potential occlusive thrombus or embolus compared to hemostatic fibrin in wound sealing clots. The composition is administered by infusion within three hours before, during or after surgery. The composition is administered by infusion at a pro-UK mutant dosage of 50 - 200 ml/hour. Preferred Catheter: The intravascular expandable catheter for delivering to a vascular site in a patient an activated, two-chain pro-urokinase (tcpro-UK) mutant comprises: (1) a catheter body having proximal and distal ends; (2) an expandable portion arranged at the distal end of the catheter body; and (3) a carrier layer arranged on a surface of the expandable portion, where the carrier layer comprises an amount of a tcpro-UK mutant effective to lyse thrombi or

emboli in contact with the expandable portion. The carrier layer is a hydrogel selected to quickly release effective amounts of the tcpro-UK mutant upon contact with a thrombus or embolus. The amount of the tcpro-UK mutant comprises 0.1-0.5 mg. The carrier layer comprises a lumen containing the tcpro-UK mutant and one or more apertures that are pressed against a thrombus or embolus to allow the thrombus or embolus to protrude into the one or more apertures, thereby contacting the tcpro-UK mutant. The carrier layer comprises activated, two-chain pro-UK mutant M5. Preferred Device: The device is a stent or suture. Preferred Composition: The composition comprises an isolated, single-chain pro-urokinase (pro-UK) mutant polypeptide, where at least 98% of the protein in the composition is the single-chain pro-UK mutant polypeptide, and an acidic excipient. Preferred Method: Preparing a pro-urokinase (pro-UK) mutant polypeptide comprises: (1) obtaining a nucleic acid molecule that encodes a pro-UK mutant polypeptide; (2) inserting the nucleic acid molecule into a pET29a expression plasmid comprising a phage **T7 promoter** and Shine-Dalgarno sequence; (3) transforming E. coli type B strain bacteria **BL21/DE3 RIL** with the expression plasmid; (4) culturing the transformed bacteria for a time and under conditions sufficient to enable the bacteria to express pro-UK mutant polypeptide; and (5) isolating the pro-UK mutant polypeptide from the transformed bacteria. The pro-UK mutant is non-glycosylated and has a molecular weight of about 45,000 daltons. The culturing comprises a two-stage fermentation. The first stage of fermentation comprises adding to a flask a cell culture diluted in sterile EC1 medium and growing the culture at about 34 to 37 degrees C for at least about 10 hours with agitation to form a seed culture, where the cell culture comprises a glycerol suspension of an LB culture of the transformed bacteria and containing a sufficient amount of kanamycin. The second stage of fermentation comprises: (1) adding the seed culture to a fermenter; (2) maintaining the pH in the fermenter at about 6.8 to 7.2; (3) maintaining the dissolved oxygen concentration in the culture medium at about 35 to 45% of air saturation; (4) maintaining the temperature of fermentation at about 34 to 37 degrees C; and (5) adding to the fermentor a nutrient feeding solution comprising one or more sugars when all glucose initially present in the fermentor at step (1) is consumed following the equation V = Vo e18t. V = is volume of feeding solution added (ml/h); Vo = is 1/100 of the starting fermentation medium (ml); and t = is time of fermentation after the start of the feeding phase (hours). The expression plasmid containing the nucleic acid molecule is pET29aUKM5. The method further comprises preparing two-chain pro-UK mutant by passing the pro-UK mutant over plasmin bound to a substrate. The substrate is an agarose-based gel filtration medium. The method further comprises combining the isolated pro-UK mutant polypeptide with an acidic excipient. Preparing a pro-urokinase (pro-UK) mutant polypeptide comprises: (1) obtaining a transformed bacteria, where the bacteria is an E. coli type B strain bacteria **BL21/DE3 RIL** transformed with a pET29a expression plasmid comprising a phage **T7 promoter**, a Shine-Dalgarno sequence, and a nucleic acid molecule that encodes a pro-UK mutant polypeptide; (2) culturing the transformed bacteria for a time and under conditions sufficient to enable the bacteria to express pro-UK mutant polypeptide; and (3) isolating the pro-UK mutant polypeptide from the transformed bacteria.

ACTIVITY - Thrombolytic; Cardiant; Cerebroprotective; Vasotropic. No biological data given.

MECHANISM OF ACTION - Gene therapy.

USE - The pro-urokinase (pro-UK) mutant is useful in clearing a lumen of blood clots or for lysing occlusive thrombi and emboli for treating a person with symptoms of stroke or heart attack in a patient before, during or after surgery. The composition comprising an aliquot of 20 to 40 mg of a pro-UK mutant, packaged with directions is useful in administering as a bolus or by infusion to a patient exhibiting symptoms

of a stroke or a heart attack. (All claimed.)

ADMINISTRATION - The composition is administered via intravenous route. No biological data given.

EXAMPLE - No relevant examples given. (90 pages)

ACCESSION NUMBER: 2004-26514 BIOTECHDS

TITLE: Use of pro-urokinase (pro-UK) mutant in clearing a lumen of blood clots for treating a person with symptoms of stroke or heart attack or in lysing occlusive thrombi and emboli in a patient before, during or after surgery; recombinant protein production via plasmid expression in host cell for use in disease therapy and gene therapy

AUTHOR: GUREWICH V; WILLIAMS J N; LIU J; SARMIENTOS P; PAGANI M

PATENT ASSIGNEE: THROMBOLYTIC SCI INC

PATENT INFO: WO 2004093797 4 Nov 2004

APPLICATION INFO: WO 2004-US11840 16 Apr 2004

PRIORITY INFO: US 2003-464003 18 Apr 2003; US 2003-463930 18 Apr 2003

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2004-775860 [76]